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THE YUCCA PLANT, *YUCCA FILAMENTOSA*, AND THE YUCCA MOTH, *TEGETICULA (PRONUBA) YUCCASELLA* RILEY: AN ECOLOGICO-BEHAVIOR STUDY¹

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Of all the delightful treatises in the field of natural history, none, I dare say, surpasses the one by Charles V. Riley on the life-history of the Pronuba moth and its relation to the pollination of the flowers of the Yucca plant. Observations and experiments on this relationship by Riley and the botanists, George Engelmann and William Trelease, covered a period of twenty years. During that long time, they repeatedly uncovered the intricate and almost unbelievable details of the behavior of the moths at the flowers, and often the work was conducted in the presence of friends and colleagues. Their findings may be verified to-day, by any one with a flashlight, during the blooming period of the plants.

Riley published short papers from time to time as the investigation progressed, and finally put the whole story together in the 'Third Annual Report of the Missouri Botanical Garden' in 1892, under the caption, "The Yucca Moth and Yucca Pollination." The paper is charmingly written, replete with beautiful drawings made by Riley himself, and is to-day regarded as one of the classics of natural history. This treatise points out to us that nowhere else do we find such hand-and-glove interdependence of flower and insect. Neither plant nor insect could perpetuate itself without the other, for the Yuccas depend solely upon the moth for pollination, and the larval moths in turn depend solely upon the ripening seeds for food.

In order to insure the development of the seeds so that the larvae may have food, the mother moth actually packs pollen into the stigmatic opening of the pistil. By this act she also accomplishes fertilization in a very difficult flower

¹My specimens of the Yucca moth were kindly identified by Mr. August Busk of the U. S. Department of Agriculture. Other insects mentioned were identified by others whose names appear in brackets throughout this paper.

which cannot be pollinated by wind or by bees. Since the larvae eat only a small portion of the growing ovules, the plant enjoys the benefit of an ample crop of seeds.

The most striking part of Riley's paper, from the standpoint of insect psychology, is the behavior of the mothers at the flowers. However, from the standpoint of ecology and evolution, the most exciting part is the discovery that the short-lived, non-feeding moths come upon the scene simultaneously with the opening of the flowers, which also are short-lived—a perfect adaptation in time of each to the other. The origin of this adaptation has never been adequately explained. Like all problems which touch upon the fascinating subject of origins, it is difficult to investigate. I have, however, made a feeble attempt at such a study, as the later pages will show. I have also verified much of Riley's work, and in the course of observations have stumbled upon additional facts on the behavior and the ecology of both insect and plant.

BEHAVIOR OF THE MOTH

First, let us review briefly Riley's discoveries on the interdependence of moth and flower.

The adult moths appear just as soon as the flowers open. Being silvery-white, they enjoy a marked amount of color protection when within the white flowers. The flowers have the most attraction during the first and second nights of their opening, probably because their fragrance is strongest at that time. The moths, small and delicate as they appear, are hardy and are strong fliers.

The mother, when ready to oviposit, gathers a ball of the sticky pollen from the anthers. In order to do this, she uncoils her tongue over the anther and stretches out to the fullest extent her maxillary tentacles. Then, by a series of forward and backward movements of the body, she scrapes the pollen with her palpi toward the tentacles. She goes from one anther to another, sometimes to as many as four, until she has a large load. This she kneads and shapes into a ball, and holding it firmly under her chin, she runs about until she finds a flower which is suitable for ovipositing. Having found one, she seeks a favorable point on the pistil and, thrusting her lance-like ovipositor into the soft tissue of the ovary, conducts the egg to its destination. No sooner is the ovipositor withdrawn than the moth thrusts a portion of her pollen-ball into the stigmatic opening and works her head rapidly "with vigor that would indicate pleasure and purpose" in packing it down. She makes every effort to force the pollen into the tube, often using her tongue to thrust it forward into the cavity.

The one load of pollen serves for several fertilizations. Each time she deposits an egg in the pistil, she repeats this process of cramming pollen into the stigma, and this is true of any subsequent eggs which are deposited in the same pistil—one fertilization for each egg deposited is the rule.

There is no other method by which larvae hatching from these eggs are assured of food, and Nature has provided no other means of pollination for this plant but this fantastic one. The behavior of the insect is all the more impressive when one remembers that she herself gets nothing at all from the flower. In fact, she has no means of imbibing food, and yet she goes through these intricate movements solely to supply, or rather to manufacture as it were, food for her young.

Riley was highly fascinated by the wonders which unfolded before his eyes, and he says:

We have in the structures and functions which are so characteristic of this Yucca moth, admirable adaptations of means to an end There is between *Pronuba* and its food-plant a mutual interdependence which at once excites our wonder, and is fraught with interesting suggestions to those who are in the habit of reasoning from effect to cause The peculiar structure of the flower which prevents self-fertilization, though on a superficial view it strikes one as a disadvantage, is, in reality, a benefit, as the value of cross-fertilization has been fully established; while the maxillary tentacles of the female moth are very plainly an advantage to her species in the 'struggle for life'; and it is quite easy to conceive, on Darwinian grounds, how both these characteristics have been produced in the course of time from archetypal forms which possessed neither, and in reality we get a good insight into the process in studying the characteristics of other species of the family Prodoxidae. These peculiarities are, moreover, mutually and reciprocally beneficial, so that the plant and the animal are each influenced and modified by the other, and the same laws which produced the beneficial specialization of parts will maintain them by the elimination of all tendencies to depart from them.

THE YUCCA PLANT AND ITS FLOWERS

The *Pronuba* moths are on hand during the very first evening that the flowers are open, and may be seen within the blossoms during the entire blooming period. Even at the end of the season, the last and only flower remaining on the stalk may be full of them. Flowers and insects appear as if by magic at the precise moment. If they did not do so, the perpetuation of both species would be defeated. This meeting at the right time is not an occasional coincidence, but evidently is the result of long years of "give and take", "come and go", and "trial and error"—natural selection at work eliminating year after year the offspring of one or the other that came upon the scene too soon or too late. Eventually the time period of both, in hand-and-glove fashion, became fixed in heredity, and the natural selection that brought on this condition of coincidental appearance maintained them by the same methods.

Before seeking to discover what influences are responsible for this coincidental appearance, one should first know something about the flowering habits of the plant. The flowers of *Yucca filamentosa* bloom in Missouri in June of each year, and remain open for only a short time. I have compiled in the table data on various aspects of the flowers' biology for nine consecutive years, 1934 to 1942 inclusive.

Year	1st flowers to bloom on author's terrace	1st flowers to bloom elsewhere in the neighborhood	Date flowers began to decline	End of blooming period	Number of flower stalks on plants on author's terrace	Number of days plants in flower
1934	June 5	—	—	—	—	—
1935	June 17	June 15	June 30	July 12	38	27
1936	June 6	June 4	—	—	—	—
1937	June 13	June 9	June 29	July 5	70	22
1938	June 4	May 31	June 22	June 30	53	26
1939	June 11	June 7	June 28	July 2	40	21
1940	June 13	June 11	June 27	July 2	58	20
1941	June 2	May 31	June 14	June 26	105	24
1942	June 2	May 30	June 19	June 29	28	27

The table shows that the flowers bloom from 20 to 27 days each year. The first flowers opened between June 2 and June 17, and the last flowers disappeared between June 26 and July 12, inclusive. The variations of the opening and closing dates, while apparently slight, are of vast importance, since the short-lived moths must, without fail, match this blooming period by their own appearance. This leaves us wondering what causal factors have sharpened Nature's mutual adjustment to so fine a point. Is this simultaneous development due to some environmental factors, such as early or late spring, wet or dry periods, sunshine or shade? And do these conditions influence in some way the flower-bearing stalks and the insects in the earth as well? If they do, it will go well with both, but if one or the other puts in its appearance too soon or too late, both flower and insect must die without leaving progeny.

The *Yucca* plants which grow on my terrace and are somewhat shaded by trees during part of each day, I regard as growing in semi-shade. There are also a large number of plants growing in a sunny open field near by. For a number of years, I have noticed that flowers appear on the plants in the sunny location earlier than they do on my semi-shaded terrace. In the third column of the table, you may see that the flowers bloom from one to four days earlier in the open sun than they do in the semi-shade.

On the other hand, in a heavily shaded estate not far from my home, I find the *Yucca* flowers appearing still later than those on my semi-shaded terrace. For

example, in 1941 the plants on my terrace began to flower on June 2, and on June 8 had 105 stalks, 82 of which were then in flower. On June 8 I examined the stalks in the heavily shaded estate, and counted 25, none of which bore a flower. Two days later, June 10, all of the 105 stalks on the terrace bore flowers, and of the 25 plants in the heavily shaded garden, only five stalks had open flowers, and then only from one to three to a stalk. Another instance in point: On July 2, on the highway near DeSoto, Missouri, I noticed a large number of Yucca plants in a heavily shaded cemetery, all of which were in full flower; a mile down the road, however, in a sunny farmyard, a similar lot was all through flowering and all stalks bore large green seed-pods.

Thus we see the effects of three environmental situations—shade, semi-shade and sunshine—on the blooming propensities of Yucca. Sunshine and shade are undoubtedly factors, within limits of course, in regulating the appearance of Yucca flowers. Riley seems perturbed when he finds the Yuccas blooming two weeks later in Philadelphia than they do in near-by Washington. He says there appears to be some irregularity in the blooming time of these plants. Evidently, what he did was to observe one set growing in the sunshine and another in the shade. In the light of the behavior of our neighborhood Yuccas, sunlight and shade would account for the irregularity which Riley observed. He does not tell us if there was also a similar irregularity in the appearance of the moths.

THE STALK, THE FLOWER AND THE SEED-POD

Before we go into the details of the regularity and irregularity of the appearance of the moths, we must have some knowledge of the day-to-day progress of the growth of the plant, especially the development of the flower-stalks, the opening of the flowers, and the ripening of the seed-pods. These details are interesting from the standpoint of flower ecology, and have some bearing on the life of the *Pronuba* moth.

Here, as an example, are the happenings on my terrace in 1941:

May 18. The flower-stalks, light-green, tender and asparagus-like, are pushing their heads up from the center of the plants.

May 27. They are now half-grown, and number 105.

June 2. 11:30 A. M. The flower-stalks are full-grown but bear no flowers. A careful search is made about the plants for any *Pronuba* moths that may be lying in wait for the flowers to open; none are found.

Same day, 10 P. M. Some time during the afternoon, 15 flowers have opened, and these are on four stalks. An examination at 10 P. M. revealed 52 *Pronubas* crowded into them. The temperature is 72° F. No moths were lurking about the plants or buds when I examined them at noon, and now there are within the flowers 21 males and 31 females. Their distribution is as follows:

		Males	Females
2 flowers, each with	_____	1	1
1 " " " "	_____	4	4
2 " " " "	_____	0	1
1 " " " "	_____	0	5
1 " " " "	_____	2	1
5 " " " "	_____	1	2
1 " " " "	_____	1	3
1 " " " "	_____	3	1
1 " " " "	_____	4	3
Total	15 " " "	21	31

June 3, 10 P. M. Three additional stalks now have flowers, and all of these harbor moths, some of which are in copula.

June 5, 10 P. M. A total of 30 stalks now have flowers, and almost all of them contain moths. Observing them until midnight, I find them nervously walking and flying about the flowers, evidently in search of newly opened ones that may not yet have served as a repository for eggs.

June 7. A total of 53 stalks now have flowers.

June 8. A total of 82 stalks now have flowers.

June 9. A total of 98 stalks now have flowers.

June 10. All 105 stalks now have flowers.

June 14. Some of the first stalks to bloom now have dead or discolored flowers, and also small, green seed-pods are beginning to form on several of them.

June 21. The first stalks to bloom are now in complete decline, but on the others, flowers continue to open.

June 23. The flowers on about 85 per cent of the stalks are rapidly declining, and some of them bear green seed-pods two inches long, thus giving evidence that Mother Pronuba has done her work well.

June 26. Only three stalks now remain that have flowers, and these total only eight. Three of these contain moths, probably newly emerged, for their wings are clean and fresh.

June 29. Only one flower now remains open, and it has attracted to itself a Pronuba, probably, too, the last one of the summer. So here ends the season of Pronuba moths and Yucca blossoms. They have appeared on the scene simultaneously, have played their brief drama strenuously, and now are no more, but the bountiful crop of green pods filled with ripening seeds and growing larvae assures continued life to both species.

July 7. The pods are large, and the seeds within them are ripening; the larvae are feeding on the seeds and are becoming large and fat.

July 16. Some of the pods show spots on the outside. These spots of discolored tissue thinly cover the exit holes in the pod-wall which were cut by the larvae in anticipation of their escape. This bit of foresight (if it may be so called) on the part of the larvae will be discussed later.

July 27. The green pods are rapidly turning brown and becoming hard, and the spots have now given way to full-sized openings from which the larvae are escaping and dropping to the earth.

August 13. The pods are becoming very tough, and the exit holes numerous, indicating that practically all of the larvae have emerged.

August 18. During the past few days, about 75 per cent of the pods have reached full maturity and have burst open, scattering the many seeds which were not eaten by the larvae. With the ripe seeds now disseminated over the surrounding earth, and a new generation of *Pronuba* babes snugly encased in silken cocoons in the ground, the activities of the whole year, all crowded into a few days, end, and for ten months life seems to have gone out. But with the advent of another summer the flowers and insects will spring into action *simultaneously* to repeat the drama.

THE ADULT MOTH

The moths, as already stated, are to be seen within the flowers during the very first evening of their opening; they continue within the perianth during the entire blooming period of 20 to 27 days; and when one peeps into the last remaining flower at the end of the season he will be greeted by the disturbed moths flying into the air.

In the foregoing chapter the details of moth and flower relations for the year 1941 were given. Additional confirmatory notes are herewith presented.

In 1935, on June 16, there were 30 flower stalks on my terrace in bud, but a careful search about the plants revealed not one adult *Pronuba* moth. The next evening, three of the stalks bore flowers, within which 18 moths were counted. On the final day of their blooming, July 12, only one open flower remained, and crowded in it were 12 moths.

In 1937, on June 17, four days after the plants commenced to bloom, 243 flowers were counted, and the moths were estimated to number about 75; ten pairs of these were in copula.

In 1938, the first flowers opened on June 4. There were 14 of them on three panicles, and at 10 P. M. 55 moths rested within them. Some flowers harbored from five to ten, and others, of course, had none. The few remaining flowers at the end of the season, June 27 to June 30, all contained moths to the very end.

In 1939, the first flowers opened on eight panicles on June 11, and during the first evening each of them contained from one to four moths; during the last days of blooming, June 28 to July 2, there were moths in the few remaining flowers.

In 1941, on the morning of June 8, six days after the flowers had begun to open, I counted only five moths in 50 flowers. In the early afternoon, more flowers burst open, and the air was full of their fragrance. The small number of moths was perplexing, to say the least, but when I visited the flowers early that evening masses of silvery winged *Pronubas* were flying to the new flowers.

This gave me the first idea that the moths do not come up from the earth

immediately surrounding the plant and walk directly up the stalk to select a flower by the sense of sight. They are without doubt attracted to the flowers by the sense of smell, and the reason I found only 5 moths in 50 flowers was that either the flowers were so old that they had lost their fragrance, or the wind was in the wrong direction or not strong enough to carry the news to the moths lying in wait somewhere. This subject will be enlarged upon later. It is strange that the fragrance of the flower should attract these insects, since for themselves food is not their goal. The object of the quest for the female evidently is a place where she may, in ichneumon-fly fashion, deposit her eggs, and the goal for the male in the flowers can only be the presence of the female. It is a complicated situation indeed if the male responds, not to the odor of the female, but to the odor of the flower she frequents.

The moths of both sexes, easily distinguishable, are short-lived and take no food, the tongue having lost its function. (In the laboratory the males lived two to three days, and the females three to five days.) They spend their adult lives within the full-blown perianth. Mating takes place there, and the pairs remain together facing in opposite directions for from two to four hours. When disturbed, they often walk about slowly without separating.

The behavior of oviposition is startling enough, but that of gathering pollen (is any other moth known to gather pollen?) and deliberately using it to impregnate the ovaries of the plant, thereby creating—instead of merely gathering—food for her young, seems as incredible, as one author puts it, "as a tale of Munchausen."

The aforementioned behaviors are mainly biological and psychological, but equally startling are the ecological facts connected with the simultaneous appearance of the moths and flowers. Has the plant adapted the time of the opening of its flowers to the appearance of the moth, or is it the other way around? Is the coincidence that we witness the "end stage" of a long series of steps of "give and take", "come and go", in the evolution of this phenomenon?

We know little of these steps, but we may some day discover them by the historical or by the comparative study of the near relatives of *Pronuba*. To digress for a moment, various not-far-distant relatives of *Pronuba* are given to many singular and astonishing ways of making a living for themselves and their young, and perhaps *Pronuba* has come by her eccentric habits honestly. To mention but a few of the queer things that her relatives do, one may cite the clothes-moths, the wax-moths, the fur-sloth moths, the cattle-horn feeders, the owl-pellet feeders, the pigeon-trash feeders. Some lepidoptera are even parasitic on bumblebees and wasps, and one species has even gone so far as to be an internal parasite of certain Coccidae. Being unwilling to wait until a study could be made of *Pronuba*'s relatives, past and present, I proceeded to carry on experiments to see what external environmental conditions may be responsible for the delicate adjustment of insect to flower in point of time.

THE EXPERIMENTS

The larva, having completed its feeding within the Yucca pod, drops to the ground, penetrates the earth a few inches and spends the winter underground in a cocoon of silk that it has spun for itself. Late in the spring, a short time before the opening of the Yuccas, the larvae transform into pupae. The pupa is heavily armed with spade-like dorsal spines with which, at the proper time, it forces itself out of the earth. When on the surface, it speedily transforms into a winged moth ready almost immediately to pollinate the flowers.

Suspecting that the larvae may be influenced by temperature conditions, simple experiments were set up to learn what one could about it. The larvae, when full fed or nearly so, were removed from the pods and placed in tin cans with loose soil; they readily buried themselves in it. The cans were tightly covered but were aerated occasionally, and the soil was moistened four or five times during the winter.

Experiment I.—Several hundred larvae were placed in tin cans on July 28, 1937, and kept in a room during the winter where the temperature varied from 42° to 60° F. A careful record was kept of the dates the adults emerged the following spring, and are as follows:

1938		Number of moths
May	17-18	55
	19-20	21
	21-22	18
	23-24	36
	25-26	49
	27-28	5
	29-30	36
June	1	41
	3	3
	6	29
	7	8
	14	13
Total		314

The data are extremely interesting in connection with the dates of flowering of the Yuccas for that year. This period for 1938 was from June 4 to June 30, 26 days. Now we see in this table that three-fourths or more of the moths emerged too early to do the plants or themselves any good. If the moths in their natural habitat emerged in the same way, it would be woe to both insect and plant. Evidently the emergence was influenced by temperature conditions, and, as one would expect, the occasionally heated room (at least it was warmer than the outside earth in which the moths normally spend the winter) stimulated development, and the moths emerged too soon.

In 1941-1942 similar tests were made under different conditions.

Experiment II a.—This is a repetition of Experiment I, and was made solely for the purpose of serving as controls of Experiments II b and II c, to follow. The dates when the adults emerged and their numbers follow:

1942	Number of moths
May 19-20	6
21-22	3
23-24	10
25-26	22
27-28	14
29-30	12
June 1-2	17
3-4	8
5-6	8
7-8	5
9-10	4
18	1
July 1	1
4	1
Total	112

Here the conditions were the same as in Experiment I, and the dates when the 112 adults emerged coincide very nicely with those in that experiment, except for two stragglers in July.

Experiment II b.—On the same day that controls were set up in Experiment II a, another one was set up as II b, with this important difference: the cans were kept outdoors in an open barn, where the temperature was practically the same as that outside. The conditions of this test are closer to those of the natural hibernating quarters of the moths in the earth, but still are not quite the same.

The dates of the emergence and the numbers were as follows:

1942	Number of moths
June 1-2	10
3-4	16
5-6	52
7-8	43
9-10	10
11-12	4
13-14	4
15-16	2
17-18	3
19-20	2
21-22	0
23-24	3
25-26	3
27-28	2
Total	154

Thus 154 moths emerged from cans in the barn from June 1 to 28; 139 during the first half of June, and only 15 during the last half. By comparing these dates with controls kept indoors (Experiments I and I a) we see that temperature is a potent factor influencing emergence. The larvae that were kept in cans in the cold barn emerged in line with those hibernating in the earth, quite in time to pollinate the Yucca flowers.

The flowers that year were open from June 2 to 29, and the first moths to emerge from my experimental cans came on June 2. They kept emerging thus from day to day during the entire blooming period of the Yuccas, with not a

moth overstaying the flowering period by even a single day. So well did this experimental emergence coincide with the normal emergence (and also with the opening of the flowers) that when my moths were liberated near the plants they joined their comrades on the flowers, as though they were "native here, and to the manner born."

Experiment II c.—This test did not turn out well, evidently due to bad technique, but is included here more as a matter of record than for scientific yield. At the time Experiments II a and II b were started, 411 larvae, in six cans, were buried a few inches below ground near the Yucca plants. The cans were covered tightly, but either because of too much moisture or the lack of air, the mortality was enormous.²

The cans were exhumed on May 20 and examined every day for emerging adults, but only five came to the top, and all from one can—one on June 9, 3 on June 13, and one on June 25. These dates were all in line with the flowering period of the plants, and indicate at least that the conditions in the ground differed very little from those in the barn.

As shown in these experiments, the influence of temperature on emergence gives us an answer, in part at least, to the question we must ask ourselves when noting the difference in blooming time for plants growing in sunshine and in shade. How can emerging moths meet this erratic blooming behavior? Reasoning by analogy from the experiments, the answer is that when low temperature or lack of sunshine retards the blossoms, it affects likewise the emergence of the moths. A portion of ground heavily shaded by trees would take a longer time to warm up than a portion in the sunshine. This would retard the moths in the earth to an analogous extent that the shady cool environment above ground retards the flowers. By the retardation of both to a similar extent, nothing is lost in the end, and flower and insect meet and function normally.

HOW THE MOTHS COME TO THE FLOWERS

As has already been stated, no moths are to be found near the plants when they are in bud, even a day or two before opening, but moths are often abundant in the flowers during the first evening of their blooming. I have always suspected that the moths bury themselves in hibernation near the growing plants, emerge from the ground some time before the flowers open, and lie in ambush, as it were, ready to fly to them at a moment's notice. In these studies I have learned that the caterpillars wander about for some time before entering the earth, and later as adults they are attracted to the flowers (as the following details will show) by the fragrance carried on the wind.

In 1935 the first flowers, three in number, opened on June 17. It rained all day on June 18, and the temperature remained around 58–60° F., and no other buds opened. June 19, with the temperature about 60–62°, 84 flowers opened,

² The cans were filled three-fourths full of soil, but those in the room and in the barn, which could more readily be handled, were often aerated.

but up to 7 P. M., not a moth was to be seen within a perianth. When I returned at 8:30 P. M., however, I found the air filled with the silvery-winged moths flying to the newly opened blossoms, while many were already settled within them, and others were walking from flower to flower, evidently seeking something "just a little bit better." I returned again at 10 P. M., and found the flight over, and from one to four moths in more than a third of the flowers, many of them in copula. It is plain to see in this case that the temperature of the day before did not deter the moths, for it differed little during the two days, but rather the lack of odor upon the wind caused their delay. It is very interesting to note also that even though the fragrance was on the air all the afternoon, the moths waited until after dark to respond to it.

During the next few days I could not visit the flowers until 10 P. M. I found newly opened blossoms from day to day, with many moths crowded into them. But on June 25, I was on hand earlier and again saw the moths flying to the flowers as they had done a few days previously. They wasted no time hovering before the flowers, but flew directly to them with a display of much nervousness and settled into them at once. They are strong fliers for so small an insect, and with my flashlight I could see them coming from the south. It was unfortunate that I could not discover from what distance they flew.

Another bit of evidence that *Pronubas* fly to the flowers from a distance was noted in 1938. A lawn in the town was leveled and resodded. Without touching the *Yucca* plants on the place, the workmen removed several inches of surface soil, thereby destroying any *Pronuba* larvae that might be hibernating there. The plants bore an abundance of flowers the following summer, but later not a stalk among them had seed-pods.³ In 1940, they also produced flowers and also many seed-pods. The moths that effected the pollination must have flown there from the population on my premises, the nearest supply, and that about 1,100 feet away. They evidently had followed the trail of odor borne by the wind. Why did they do so in 1940, and not in 1939? My answer is, reasoning from analogy,⁴ that it is quite likely the wind was not favorable in direction or in strength for carrying the flower odor to the places where the moths were.

Riley says (l. c., p. 122): "I have often been struck with the power which the moth has of detecting isolated plants blooming for the first time remote from other plants . . . a fact which indicates that, where abundant, in addition to her ordinary more sedentary duties, she takes long reconnoitering flights."

In summary, I may say that *Pronubas* fly against the wind on the trail of the fragrance of the *Yucca* flowers, where they proceed promptly to the business of egg-laying. *Pronuba* moths do not fly at all hours of the night, but only between 8:30 and 9:30 P. M. This rhythmic periodicity is also found in certain species of fireflies and certain Saturniid moths, each species having its own set period for flight some time between twilight and dawn. The *Pronubas* often run

³ The flower stalks shrivel when the flowers are not pollinated.

⁴ The sex attraction and rhythmic periodicity of Saturniid moths. Acad. Sci. St. Louis, Trans. 26:81-221. 1929.

restlessly from flower to flower; when this occurs, it is because there are too few newly opened flowers at hand, and the old ones have lost their attractiveness. I have frequently seen a moth inspect several flowers before selecting one in which to oviposit. Riley says that the stigmatic opening closes when once eggs are deposited in the pistil; perhaps this is a sufficient signal for the moth to seek a favorable place to oviposit elsewhere.

Unlike other moths, *Pronubas* are not attracted to light. The lighted windows of my home, only 40 feet from the terrace, have never attracted them. Only when they escape in the laboratory, where the dazzling light is very near, do they circle around the electric bulb in a confused manner.

THE LARVAE

Like the adult *Pronuba*, the larvae are quite hardy and can stand a lot of rough handling. Riley likewise found them so, for he says:⁵ "It is the hardest larva I have had to do with, and will not only repeatedly mend its cocoon when it is cut or torn, but when extracted from it, will survive for months if kept in a tight vessel." More than that, I find that the panicles of seed-pods may be cut from the plant and transported in the automobile for long distances. During the shake-up, many larvae fall from the pods and may later be picked up from the floor of the car. When the more mature ones of these are placed in cans of loose dirt, they will develop into normal adults.

The tiny, white, newly hatched larvae feed upon the white ovules. As the seeds become larger and darker, the larvae too grow larger, fatter and more colorful, and finally when the caterpillars reach full growth the color is red, tinged with green.⁶ They eat the tender centers of a row of tightly packed seeds, destroying from 18 to 25 in the process. The tough rims of the seeds are not eaten, but serve (closely packed together as they are) as a wall of the cell-like domicile while the larvae continue to eat their way through the compact row. In addition to being closely packed, the seeds are held together by strands of silk spun by the larvae, as well as by bits of excrement pushed to the far end of the tunnel. This makes a comfortable "cocoon" and is so tightly held together that the whole set of otherwise loose seeds may easily be removed as one mass.

In the darkness of its cell, the larva grows while it eats its way through its food-mass and enlarges its tunnel. But toward the end of its career, it exhibits a bit of behavior that seems to bespeak purpose as much as the adult action in pollination. When nearly mature, the caterpillar interrupts its feeding long enough to cut a hole in the outer wall of the pod to permit its later escape. It does this while the pod is still tender, and its jaws can crush the green tissue of the wall. If the caterpillar waited too long to bite this hole, the walls would be found to be too tough, and the insect would be entombed. This job neatly done, it resumes

⁵ Sixth Rept. State Entomol. Mo. pp. 131-135. 1874.

⁶ One sometimes finds full-grown caterpillars that are of a beautiful amethyst-green hue. In one lot of 80, three such were found. When they were brought to maturity, the adults differed in no perceptible way from the reddish-green ones.

its feeding.

This precaution, which so resembles foresight, is not the only commendable item of its behavior. When cutting this hole through the wall of the pod, it stops short when it reaches the thin green outer skin. Thus the hole is concealed from the outside, yet is easily broken when the larva is ready to emerge. This concealment of the hole might evade enemies, for birds occasionally break open the pods. But Riley says that the open holes permit moisture to enter, causing a growth of fungus which might be detrimental to the larvae.⁷

While normally the caterpillar eats the tender centers of its row of seeds, when it cuts the exit-hole for future use, it must, in order to reach the pod-wall, bite its way out through the outer edges of the row of seeds; also it must turn squarely at right angles to its habitual course. After these sharp digressions from its usual quiet life, it goes back to feeding. The little discs of skin, like tightly stretched drum-heads, covering the holes, often turn brown while the pod is yet green, and are tell-tale landmarks that point to a fat larva just beneath. The holes are small, and the fat larvae have to struggle to push through them when escaping. They do not just fall out of the holes, but wriggle through in what appears to be a painful ordeal, at last breaking the thin, outer skin of the pod as they come out.

After the larvae have dropped to the earth, they walk about for some time before burrowing into it. Those in the laboratory, when placed on loose soil, wandered about for several hours before crawling down into it. It was formerly thought that the larvae, falling from the pods, enter the ground near their own plant; but seeing the larvae busily crawl about in the cans of earth, and also later seeing the adults fly to the flowers from a distance, I concluded that the larvae travel some distance from the plant for hibernation.

Riley says that the larvae penetrate the ground five or six inches, but in my tin cans they went down into the loose soil from one to three inches. They spend the winter underground, and in the late spring they transform into heavily spined pupae. At a later propitious moment they work their way out of the ground, shed the horny covering, inflate the silvery wings, and are ready for the business of reproduction when the first fragrance of *Yucca* flowers permeates the air.

If the larvae are needed for experimental purposes, the pods must not be gathered too early, or the larvae will be underfed; on the other hand, if one waits too long, the larvae will have escaped into the ground. They should be gathered, as near as possible, just when they have finished feeding; and the tell-tale brown spots on the outside of the full-grown green pods indicate that feeding is nearly

⁷ It is interesting to note that the larvae of the bogus *Yucca* moth, which has a common ancestral origin with *Pronuba*, behave in a similar fashion. They feed on the pith of the flower stalk, but before spinning a cocoon eat a passage-way to the outer covering of the stem. However, they leave intact the thin membrane on the outside, through which later as adults they escape. Those which emerged from dried stems gathered hereabouts proved to be, according to Mr. Carl Heinrich, *Prodoxus quinquepunctellus* Chamb., which he states is a synonym of *P. decipiens*.

over. If the caterpillars are taken before, they are mature, the mortality will be great. In a lot of 300 larvae gathered too soon, there was a mortality of 95 per cent in my cans, while under similar conditions, in a lot carefully selected for complete feeding, 48 out of 50 larvae transformed into normal adults.⁸

Dates of the exodus of the larvae may vary slightly in different localities and in different years in the same locality. However, at Kirkwood, pods on about July 15–25 contain full-grown larvae, while perhaps ten days later, the holes will be open and the larvae gone. If the panicles are left on the stalk, the pods naturally dehisce. If they are brought into the laboratory, they harden prematurely, and this before the larvae within can provide the escape-holes. The larvae are then prisoners, but they spin cocoons around themselves in their tunnels of half-eaten seeds and spend the winter in that way. A lot was discovered one spring, after having spent the winter within the pods in my cold barn, and they were found to be in good condition. Later all became normal adults.

It is amazing that so few *Pronuba* moths are parasitized. With several thousand developing in the laboratory, not one parasite issued from them. It is not due to the fact that the larvae are distasteful, for I have fed dozens of them to *Polistes* wasps, which in turn fed portions of the meat to their larvae. They were accepted as food by larval ant-lions also.

The larvae are generally free from enemies, excepting for a bird occasionally breaking into a pod, or a mouse eating the larvae along with the seeds in the laboratory. However, Riley has found ants destroying the larvae in the ground.

The larvae live and grow in these apparently air-tight pods, and the number per pod varies. There is no relation between the size of the pod and the number of insects feeding within it. In 1937, near the end of the season I gathered 10 panicles bearing 316 pods. Dissecting the pods, I made a count of the larvae within them, with the following results:

Number of larvae in each pod	Frequency	Total number of insects
0	3	0
1	12	12
2	19	38
3	48	144
4	60	240
5	64	320
6	54	324
7	16	112
8	24	192
9	8	72
10	7	70
11	0	0
12	1	12
Total	316	1536

⁸ The tin cans were about three-fourths filled with loose earth and covered with tin lids, but they were aerated and lightly moistened during the season.

The 316 pods harbored 1,536 larvae, or an average of nearly 5 per pod. But it is interesting to note that more than two-thirds of the pods harbored from 3 to 6 larvae. In an extreme case, one pod had 12 larvae, and in this pod every seed was destroyed. In each of the 15 pods containing 9 and 10 larvae, only a few seeds (from 6 to 15) remained uneaten. In most of the other pods, there remained hundreds of good seeds ready for dissemination.

An interesting item in the table is the fact that three pods containing seeds had no larvae in them. This was to be expected, since these three pods bore no constrictions. Riley has shown that the deposition of the eggs in the pistil is responsible for the constrictions in the middle of the maturing pod. If the constriction is slight, only one or a very few larvae is likely to be found within the pod; if it is deep, many may be expected. Riley was able completely to eliminate the constrictions by pollinating the flowers by hand.

Riley found also, and my observations substantiate this, that no other insect is able to pollinate the *Yuccas*, since pollination requires that the sticky pollen be tightly packed in the stigmatic opening.

Of the 316 pods here examined, only 3 bore no larvae, and these had no constrictions. In the light of Riley's observations, my only explanation is that either the mother's ovaries were depleted, in spite of which she packed the stigmatic opening with pollen, or her instinct went so far astray that she packed her little bundle of dynamite into the stigma but omitted to place the egg in the pistil. Similar miscarriages of instinct often occur among the solitary wasps, where plentiful food is provided for the young and the egg is not deposited.

INSECTS OTHER THAN PRONUBA TAKEN ON THE PLANTS

Riley presents a list of several other insects which are to be found about *Yucca* flowers and plants.⁹ He found positively that these insects had no hand in the pollination of the flowers; that office is performed by *Pronuba yuccasella* alone. I have found other insects about the plants, and I also have ascertained that these have no part in the transfer of pollen. A list of insects and their behavior follows, and I should like to mention that only three of my records are the same as Riley's; these three are marked with an asterisk.

BEETLES¹⁰

**Carpophilus melanopterus* Ev. [E. A. Chapin]. These beetles were present each year, and sometimes six or eight were to be found in one flower. Sometimes they shared a flower with several *Pronubas*. They were present during the entire blooming period of each year, but especially they were noticeable in the very first flowers on the night of their opening, and often did damage by eating portions of the pistil or by biting their way into the very heart of the unopened bud.

Obrium maculatum Oliv. [W. S. Fisher]. Only one beetle of this species was taken; it was on the outside of the flower on June 25, 1937.

⁹ Footnote in Fifth Rept. Insects Mo. p. 154. 1873, and in Amer. Assoc. Adv. Sci. 29:626. 1880.

¹⁰ Names in brackets are those of persons who identified the insects.

Anthrenus trifasciatus Melch. [H. S. Barber]. Only one specimen of this rove-beetle was taken; it was inside a flower, June 25, 1937.

**Chauliognathus pennsylvanicus*. The Pennsylvania soldier beetle was found each year about the leaves and within the flowers.

Coccinella novemnotata Hbd. [E. A. Chapin]. During the blooming season of 1935, many of these beetles, some of them in copula, were on the flowers. They were present on the stalks before the flowers opened, and remained through the blooming season.

Coleomegilla fuscilabris Muls. [E. A. Chapin]. One beetle seen on an unopened flower bud.

Trichiotinus piger F. [E. A. Chapin]. Only one of these Scarabidae was taken; it had its head deeply buried in a Yucca flower.

PLANT-LICE

Plant-lice, *Aphis rumicis* L. [P. W. Mason], are always abundant each year on the green flower stalks, although some years their numbers are much reduced by the aphid-lions. They usually collect on the bracts before the buds open and remain on them long after the white petals have fallen, often damaging the unopened buds. They feed on the juices of the plant, and appear to be just as abundant at the beginning of the season as at the end. They are often attended by the ant, *Formica fusca* var. *subserica* Say [M. R. Smith].

ANTS

When the tender flower stalks thrust up their asparagus-like heads, and later when the flowers are in bloom, one may often find aggregations of *Formica fusca* var. *subserica* Say upon them. They no doubt have been attracted to the plants by the plant-lice, but they have also been seen licking the exudations from the unopened flower buds.

Two other species of ants, *Monomorium minimum* Buckley [M. R. Smith] and *Penolepsis* (*Nylanderia*) *pavula* Magr. [M. R. Smith], were often seen on the flower-stalks, but what their interests on the plant were has not been ascertained.

APHIS-LIONS

The aphid-lions, *Chrysopa nigricornis* Bur. [A. B. Gurney], appeared in great numbers during certain years. At such times the plant-lice were greatly reduced. The females have often been observed depositing their stalked eggs on the plants at night.

HONEYBEES

*Honeybees, *Apis mellifica*, are always to be found about the flower-stalks, but they are seldom inside the blossoms. They usually content themselves with gathering the excretions on the outside of the base of the flowers. When they lay up the invisible excretions with protruding tongue, their abdomens pulsate rhythmically.

FLIES

Syrphus torvus O. S. [C. T. Greene]. These flies were often seen in company with the honeybees, lapping the exudations at the base of the flowers and also from the outside of the petals. Sometimes they fall prey to the flower spider, *Misumenops asparatus* Hentz. [E. B. Bryant], which often hides among the petals.

Allograpta obligua Say [C. T. Greene]. This fly was taken from the jaws of the above-mentioned spider in the center of a flower.

LEPIDOPTERA

Peridroma margaritosa Haw. [Carl Heinrich]. A caterpillar of this Noctuid species was seen eating into a flower-bud.

BUGS

Lygus pratensis oblineatus Say [H. G. Barber]. Occasionally a bug of this species was seen feeding on an unopened flower-bud.

Lopidea instabilis Reut. [H. G. Barber]. Seen occasionally feeding on flower-buds of the plant.

Leptocoris trivittatus Say [H. G. Barber]. A few nymphs taken from the plants during the blooming period in 1939.

Halticotoma valida Reut. [H. G. Barber]. This insect, known as the Yucca bug, has appeared on the plants on my terrace in such numbers during certain years as to injure them and reduce the number of flower-stalks. During 1939, only 40 flower stalks appeared (against 105 in 1941), and the flowers on each stalk were very few. Not one blossom was free of the bugs. Their sucking also produces numerous spots on the leaves, and not a leaf was free of these spots.

This enormous population in 1939 was evidently due to my having neglected to remove the fallen leaves that had accumulated about the plants for two years. The plants, however, recovered quickly when the accumulated debris was destroyed and the bug population thus reduced. After this had been done in 1940, the flower-stalks in 1941 numbered 105, all of which flowered heavily. The infestation appeared only on my neglected plants; other plants in the neighborhood were not noticeably infested. The bugs evidently find favorable winter quarters among the dead leaves, and spend their entire summer lives on the Yucca leaves, for in 1939 they were as abundant in October as they were in May.

INTERPRETATIONS OF PRONUBA'S BEHAVIOR

From the standpoint of comparative psychology, the behavior of *Pronuba* is of outstanding importance, and many students of behavior have sought in one way or another to explain the thorny problem of her actions. For example, McDougall¹¹ captions his discussion of the subject "Purely Instinctive Behavior," yet he is far from clear in throwing any light on the matter of the origin of these instincts. After describing the behavior of the moth at the flower, he says:

Nature has so constituted the moth that she performs this cycle of nicely adjusted actions, essential to the continuance of the species, shortly after emerging from the chrysalis, when

¹¹ Outlines of psychology. pp. 74, 76. 1923.

she cannot have acquired any knowledge of the flower or of her grub and its needs. This is a fine example of the working of a chain instinct. Each step in the train of action brings the moth into a new situation in which new stimuli affect its sense organs. Why not be content to suppose, with the mechanists, that each step is simply a reflex action to some new stimulus Consider a single step in this behavior, the placing of the egg in the one position in all the world where it can develop, this is among the ovules of the flower. Even if we assume that odor emanations from the ovules exert some tropic influence on the moth, it is obvious that this will not suffice to determine the placing of the egg in the right spot. That can be effected only under the guidance of a multitude of simultaneous and successive sense stimuli; and these must be not merely summated but rather synthesized and related to an appreciation of the shape of the parts of the flower concerned. In other words, the response of the moth to the flower is a perceptual response, not a mere reaction to a stimulus.

When one tries to find the meaning of "perceptual response" in his book, one is referred in the index to "Perceptual response to instinct" on page 99, but there we read the meaningless jargon which runs as follows:

Instinctive activity is normally initiated by an activity of perception, more or less complex; the capacity for this activity is given in the innate constitution of the animal, and is an essential part of the total instinctive disposition (or instinct) as the capacity to execute the train of bodily movements which catch our eye.

It seems to me, however, that if the action of the moth is a *perceptual response*, then it is not an instinctive one, but rather more or less akin to discriminating behavior. Fearful of crediting *Pronuba* with psychic attainments of too high an order, McDougall, in my opinion, gets nowhere in his attempt to explain the insect's behavior.

Wells, Huxley and Wells likewise take a shot at *Pronuba*'s behavior, and are likewise parsimonious in interpreting her actions in ovipositing. They say:¹²

The impossibility of there being knowledge behind instinct is perhaps most prettily illustrated in the well-known case of the yucca plant and its moth, *Pronuba* The association is one of mutual benefit, a reproductive symbiosis; the action of the female moth in putting the ball of pollen on the pistil seems admirably purposeful, just as her care not to kill the goose that lays the golden eggs, by only introducing three or four grubs into each flower-capsule, seems admirably calculated. But when we reflect that the mother moth dies before the seeds mature, and that the moths of the next generation have never seen a yucca in flower before they began their business of pollen-gathering and egg-laying, it becomes obvious that foresight and reason can play no part in the instinct—quite apart from the fact that experiments have decisively shown that no insect is capable of drawing such conclusions as the moth would have to draw if it were really being intelligent on the facts presented to it. We have no more right to suppose that the moth is being purposeful and intelligent in its actions than the yucca is being purposeful and intelligent in growing a pistil with a cup at its tip to receive the pollen; or, to confine ourselves to the moth, we have no more reason to find proof of intelligence in its actions in putting the yucca pollen in the proper place than in its growing the special appendage with which to manipulate the pollen.

Their parsimony goes still further when they say an instinct "is the outcome of the animal's nervous construction, as the leg and its working is the outcome of its mechanical construction. It is a bit of nerve-clockwork."

The statements by Wells, Huxley and Wells have the advantage of logic, but also the limitations of laboratory study. The authors go as far as they can in the generality that an instinct is the outcome of the animal's nervous constitution (which may or may not be true, because for all we know, the animal's nervous

¹² The science of life, p. 1153. 1929.

constitution may be the outgrowth of its psychic life, just as the mechanical construction of its leg may also be the outcome of its movements), but that is beside the point, since they have said nothing to bring us any nearer to an understanding of how all this came about.

Riley, on the other hand, takes a more magnanimous view of Pronuba's psychic qualities. He goes quite far in his anthronomorphic explanation, which is probably the result of his having spent twenty years observing the behavior of these silver-winged moths in the field:

The pollen grains would not adhere by chance to the rolled-up tentacles, and we have seen how full of purpose and deliberation Pronuba's actions are. It may be that all her actions are the result merely of "blind instinct", by which term proud man has been wont to designate the doings of inferior animals; but no one can watch her operations without feeling that there is in all of them much of purpose Nor can I see any good reason for denying these lowly creatures a degree of consciousness of what they are about, or even of what will result from their labors. They have an object in view, and whether we attribute their performances to instinct or to reason depends altogether on the meaning we give to those words. Define instinct as "congenital habit" or "inherited association" or, as I prefer to characterize it, as *the inevitable outcome of organization* [italics Riley's] and most of the doings of the lower animals may justly be called instinctive; but the instinctive and reasoning faculties are both present, in most animals, in varying proportion, the last being called into play more especially by unusual and exceptional circumstances, and the power which guides the female Pronuba in her actions differs only in degree from that which directs a bird in the building of its nest, or which governs many of the actions of rational man.

Coquillett, to quote from Lovell¹³, is even more positive than Riley, for he regards the behavior of Pronuba as a purely intelligent act, saying: "There appears to be no doubt that she is in possession of the fact that unless she did thus pollinate the flower, there would be no seed pods for her offspring to live on."

Riley, as you have seen, grants to Pronuba a higher degree of psychic ability, and he does so evidently because he repeatedly observed how full of purpose and deliberation her actions are; but when he, as well as Coquillett, credit the moths with a consciousness of what will result from their labors, they merely indulge in a guess, for who can know what goes on in the heads of these creatures!

However, one must admit that there are in the insect world numerous analogous cases where the participants likewise act as if they knew, and knew very well, what would be the end result of their labors. Whenever I see Pronuba deliberately pounding the pollen into the stigmatic opening, other brilliant behaviors come to my mind. Who can deny, for instance, that the Empis fly does not realize to what purpose he dances before the female with the marriage offering of a captured may-fly which she is to suck during the process of mating; or who will doubt that the queen bumblebee has some consciousness as to what purpose she broods her eggs when, hen-like, she keeps them warm day and night until they hatch; or the male butterfly of the genus *Belenois*¹⁴ to what purpose he strokes the wings of the desired mate; or the saw-fly, *Perga lewisii*¹⁵, the end for

¹³ The flower and the bee. p. 144. 1918.

¹⁴ Carpenter, G. D. H., A naturalist on Lake Victoria. p. 223. 1920.

¹⁵ Carpenter, G. D. H., The biology of insects. Chap VIII. 1928.

which she strives when she watches over the eggs and later follows the young about as they feed, often covering them with her body to shield them from enemies and protect them from the sun; or the earwig, *Anisolabis mortima*,¹⁵ when she cleans her eggs by rolling them in her mouth, and watches and guards them, as well as the young, when they are born; or does the bug, *Aepophilus bonnarei*,¹⁵ when she gives the warning taps with her antennae which sends her young scampering for cover; or certain agricultural ants when they carry in their jaws on their marriage flight a pellet of fungus to start new gardens? And I cannot but recall my own observations on the intricate behavior of cockroaches,¹⁶ in depositing and concealing their egg-cases. Many other examples could be cited.

But even if it is true that *Pronuba*'s behavior is purely instinctive, we must admit that it could not possibly have always been so, for even an instinct must have had a beginning at some time. There is a first time for everything, and in the vast sweep of evolution, somewhere, sometime, certain especially endowed individuals, perhaps spurred to frantic exertion by some life-and-death stress, made unusual use of their faculties and adopted new ways with the flowers. The fact that a species performs a highly complicated and effective course of action, even though that course of action may now have become crystallized into instinct, points clearly to a line of progenitors who were versatile and were not afraid to try something new. It is an especially significant fact that relatives of this moth display an astonishing variety of outlandish accomplishments (mentioned elsewhere) which would justify our contention that the little *Pronuba* came from an "Edwards family" and not a "Jukes" in the insect world.

One may say in conclusion that if we wish to accord to present-day *Pronubas* a grain of intelligence, it is with the understanding that a great part of their actions are based on a well-developed set of instincts which were probably acquired bit by bit through the ages. On the other hand, branding their behavior as instinctive does not by any means preclude an ability occasionally to mix with it a bit of original variation, or a grain of something akin to intelligence. It may even require a modicum of intelligence to know when and where to make the best use of an equipment of instincts.

THE EVOLUTION OF THE INTERRELATIONSHIP

Both *Yuccas* and *Pronubas*, says Dr. William Trelease¹⁷, are undoubtedly of recent geological origin; and the progenitors of the *Yucca* originally had spreading stigmas, and were also slightly entomophilous flowers pollinated by hymenoptera, diptera, or lepidoptera, which were attracted by the secretion of the septal nectar glands.

With the consolidation of the stigmas, however, insects visiting the flowers for this nectar became inefficient pollinators, as may be seen when such insects enter the flowers of the existing *Yuccas* for the little nectar that is still produced; hence, with an economic reduc-

¹⁶ See article, "How the cockroach deposits its egg-case; a study in insect behavior." *Ann. Ent. Soc. Amer.* 36:221-226. 1943.

¹⁷ *Ann. Rept. Mo. Bot. Gard.* 4:217. 1893.

tion of the secretion of these glands, may have come an addition to their function to that normally borne by the stigma, in an increase in its secretion, so that the visitors, laden with pollen unconsciously accumulated while on the flower, should further visit the stigma on which some of their burden might be rubbed while they were feeding. During this stage of its evolution the plant appears to have proved especially attractive to some small moth, perhaps fond of nectar, and with phytophagous larvae, which is to be regarded as the progenitor of the *Pronuba*. . . .¹⁸

Riley, too, agrees that *Pronuba* and *Yucca* have arisen from simpler forms, for he says.

The peculiar structure of the flower . . . prevents self-fertilization; . . . while the maxillary tentacles of the female moth are very plainly an advantage to her species in the "struggle for life"; and it is quite easy to conceive, on Darwinian grounds, how both these characteristics have been produced in the course of time from archetypal forms which possessed neither. . . .¹⁹

Since the structure of the insect has undoubtedly changed in the course of evolution, it is quite obvious that psychic changes have likewise occurred, and perhaps, after all, the brain and the mind of the free-flying *Pronuba* have played a more important role in the evolution of this singular relationship than has the brainless, immovable plant, which at most could have played only a passive role.

One can hardly assume that this mutual adaptation was a general merry-go-round process through the ages, each contributing equally to the other. The flower in the shadowland of its evolution could do no more than sway in the wind and abide its time, even as it does to-day. It had no choice in the selection of insects to perform the marriage rite, and could do no more than shed its fragrance on the passing breeze, and thus advertise its charms. The quality and condition of its charms, no doubt, varied over countless millenniums, and the insect was often compelled to choose or consciously select from among several variants.

The flower's important charms, in so far as *Pronuba*'s behavior is concerned, are the stigma, the pollen, and the pistil, and from diversifications in these it had to select, for example: the stigmatic opening best suited to its pollen-pounding tongue; the pollen, dry, wet or moist, best suited to the carrying capacity or to the manipulating ability of its jaws; and the pistil best suited to the penetration of its peculiar ovipositor. In short, the blossom is selected by the insect and not the other way around. *Pronuba* has, in hammer-and-anvil fashion, hammered, let us say, the *Yucca* flower into what it is to-day, and the insect itself, in so doing, has undergone numerous changes—psychological and otherwise.

The *Yucca* apparently, as already suggested, has played but a minor part in the creation of the novel relationship, but a very important part, nevertheless—for if there were no *Yucca*-like flowers ever, there would not be (nor could there possibly be) the unique creature which we know to-day as *Tegeticula* (*Pronuba*) *yuccasella*.

¹⁸ *Ibid.* p. 219.

¹⁹ *Ann. Rept. Mo. Bot. Gard.* 3:126. 1892.

A REVISION OF THE CENTRAL AMERICAN SPECIES OF SMILACINA*

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I. INTRODUCTION

The genus *Smilacina* was established by Desfontaines¹ in 1807, and was based on *Convallaria racemosa* L. Linnaeus², in the 'Species Plantarum', first edition, had included under the generic name *Convallaria* eight species. All but one, *C. majalis*, were later segregated and referred to *Polygonatum*, *Smilacina*, and *Maianthemum*. The assemblage of these plants in one genus by Linnaeus was based principally on the spotting of the young berries, a character which is more or less common to the entire group.

Desfontaines, considering several other characters, divided *Convallaria* into four distinct genera: *Convallaria*, *Polygonatum*, *Smilacina*, and *Maianthemum*. The distinguishing features were found in the nature of the flowers and in the manner in which they are borne on the stem. *Smilacina* was distinguished because of its terminal inflorescence, the division of the perianth into six separate segments, and the star-shaped corollas.

Five species of *Smilacina* were described by Desfontaines, two of which have been collected in Mexico, namely, *S. racemosa* (*Convallaria racemosa* L.), the type species of the genus, and *S. stellata*. Of the remainder, *S. ciliata* apparently is a synonym of *S. racemosa*, *S. trifolia*, a boreal species of both hemispheres which does not enter our range, and *S. umbellata*, which becomes *Clintonia umbellulata* Michx. Bertoloni³ added a new species in 1840, *S. flexuosa*, and was followed two years later by Martens and Galeotti⁴, who described three additional species and one variety: *S. macrophylla*, *S. scilloidea*, *S. paniculata*, and *S. scilloidea* var. *acutifolia*. *Smilacina amoena* was published by Wendland⁵ in 1850.

J. G. Baker⁶ was the first to compose a monograph of this group, but used the earlier name *Tovaria* Necker. In this work four new species appeared: *T. thyrsoidea*, *T. laxiflora*, *T. nervulosa*, and *T. Salvini*. The first three species are regarded in this revision as synonyms of *S. paniculata* Mart. & Gal., whilst the

¹ Desf. Ann. Mus. Par. 9:51, t. 9. 1807.

² Linn. Sp. Pl. ed. 1. 315. 1753.

³ Bertol. in Nov. Comm. Acad. Bonon. 4:411, pl. 39. 1840.

⁴ Mart. & Gal. in Bull. Acad. Brux. 91:387-388. 1842.

⁵ Wendl. in Otto & Dietr. Allg. Gart. Zeit. 17:137. 1850.

⁶ Baker in Jour. Linn. Soc. Bot. 14:564. 1876.

* An investigation carried out at the Missouri Botanical Garden and submitted as a thesis in partial fulfillment of the requirements for the degree of master of science in the Henry Shaw School of Botany of Washington University.

fourth apparently is a color variety of *S. amoena* Wendl.

In his treatment of *Smilacina* for the Botany of the 'Biologia Centrali-Americana,' Hemsley⁷ merely abstracted the revision of Baker, and making the necessary transfers from *Tovaria* recognized seven species: *S. flexuosa*, *S. laxiflora*, *S. nervulosa*, *S. paniculata*, *S. Salvini*, *S. scilloidea*, and *S. thyrsoides*. Like Baker, Hemsley was unable to place *S. paniculata* satisfactorily, considering it as identical with *S. amoena*, and thought *S. macrophylla* to be a synonym of *S. scilloidea*.

Since 1884 the only new species of *Smilacina* to be published from Central America and Mexico is *S. Gigas* Woodson⁸, from Panama, which probably is only a giant phase of *S. paniculata* Mart. & Gal.

Because of its popularity and general use, the generic name *Smilacina* was conserved by the International Botanical Congress of Brussels (1910), since it is antedated by three previous genera: *Vagnera* Adans.⁹, *Tovaria* Neck.¹⁰, and *Polygonastrum* Moench¹¹. Later generic synonyms include *Sigillaria* Raf.¹², *Stylandra* Raf.¹³, *Asteranthemum* Kunth¹⁴, *Jocaste* Kunth¹⁵, *Medora* Kunth¹⁶, and *Neolexis* Salisb.¹⁷, all of which fall readily into synonymy.

II. MORPHOLOGY AND GENERIC RELATIONSHIPS

Roots.—The roots of *Smilacina* are borne either at the nodes or all over the surface of the underground rhizome. They are either simple or shortly branched, closely placed to one another and forming a dense mass as in *S. amoena* var. *Salvini*, or more loosely clustered at the nodes as in *S. scilloidea*. They are relatively slender and sometimes very long, measuring a foot or more in length. They are usually covered with a dense felt of persistent root hairs.

Rhizome.—The rhizome varies considerably both in size and in some external characters such as type of branching. But due to the difficulty in pressing these large underground stems, they are seldom collected and not much is known of their variability or taxonomic importance. They are essentially simple or branched, and apparently reach relatively great lengths in most species. They may be thick and very fleshy as in *S. amoena* var. *Salvini*, or quite slender as in *S. scilloidea*. The rhizome of *S. racemosa* is very knotty due to the closely budding upright shoots, while those of *S. scilloidea* and *S. stellata* are rather smooth and slender, since there is less budding.

⁷ Hems. Biol. Centr.-Am. Bot. 3:367-368. 1884.

⁸ Woodson in Ann. Mo. Bot. Gard. 27:270. 1940.

⁹ Adans. Fam. Pl. 2:496. 1763.

¹⁰ Neck. Elem. 2:190. 1790.

¹¹ Moench, Meth. 637. 1794.

¹² Raf. in Jour. Phys. 89:261. 1819.

¹³ Raf., loc. cit. 102. 1819.

¹⁴ Kunth, Enum. Pl. 5:151. 1850.

¹⁵ Kunth, loc. cit. 154. 1850.

¹⁶ Kunth, loc. cit. 155. 1850.

¹⁷ Salisb. Gen. Pl. Fragm. 64. 1866.

Stems.—The stems are erect, unbranched, and typically herbaceous, arising from the terminal bud of the rhizome. The basal portion usually is clothed with very thin, scale-like, sheath leaves from the terminal bud of the rhizome. The height of the stem varies from 0.2 to 3 m., and may be relatively constant as in *S. amoena*, *S. racemosa* and most of the other species, or extremely variable, as in *S. paniculata*. In the living state the color varies from green to somewhat reddish or purplish at the base or in the inflorescence.

Leaves.—The leaves are alternate, and may be either sessile or petiolate. They vary in shape from narrowly lanceolate to broadly elliptic. In length they measure from about 3 to 30 cm. with the same extremes in width. All have longitudinal primary veins, some of which branch from near the leaf-base and are more prominent than the others. The number of these principal veins is rather constant for certain species, but is often difficult to observe. The great majority of the species bear glabrous leaves, but in some plants of *S. flexuosa*, and generally in *S. stellata*, there is an inconspicuous pubescence. The leaf characters vary considerably in the genus, and often even in the same species. It would be difficult to delimit the species by using such characters because of their inconsistency amongst individuals clearly conspecific in other details.

Inflorescence.—The inflorescence of *Smilacina* is terminal, or very rarely with axillary inflorescences in the axils of the uppermost leaves. Bracts usually are apparent only at the lower nodes. The best characters for the distinction of the species are found in the form of the inflorescence. These characters are shown in the diagrams of fig. 1, which also is a good indication of the probable phylogeny within the genus. There apparently is an evolutionary series from a diffuse panicle to a typical raceme, resulting through the suppression of secondary peduncles.

In *S. paniculata* and *S. racemosa* the inflorescence is a typical panicle, the secondary peduncles being extensive and many-flowered. The pedicels are relatively short, scarcely longer than the perianth and occasionally even somewhat shorter. In *S. amoena* the paniculate structure is less obvious, and the secondary peduncles have become shortened and few-flowered; and the pedicels are much longer than the perianth.

The secondary peduncles have become completely reduced, or rarely only slightly manifest in *S. flexuosa*, *S. scilloidea*, and *S. macrophylla*. The chief indication of a previous compound nature is found in the clustering of the flowers at the nodes of the primary peduncle. In *S. flexuosa* the rachis is conspicuously flexuous or geniculate, and the flowers are borne in groups of twos, threes, or occasionally fours. In this species the pedicels elongate very conspicuously after anthesis. The rachis is essentially straight in *S. scilloidea*, and is scarcely stouter than the pedicels, which occur usually in twos. In *S. macrophylla* the inflorescence

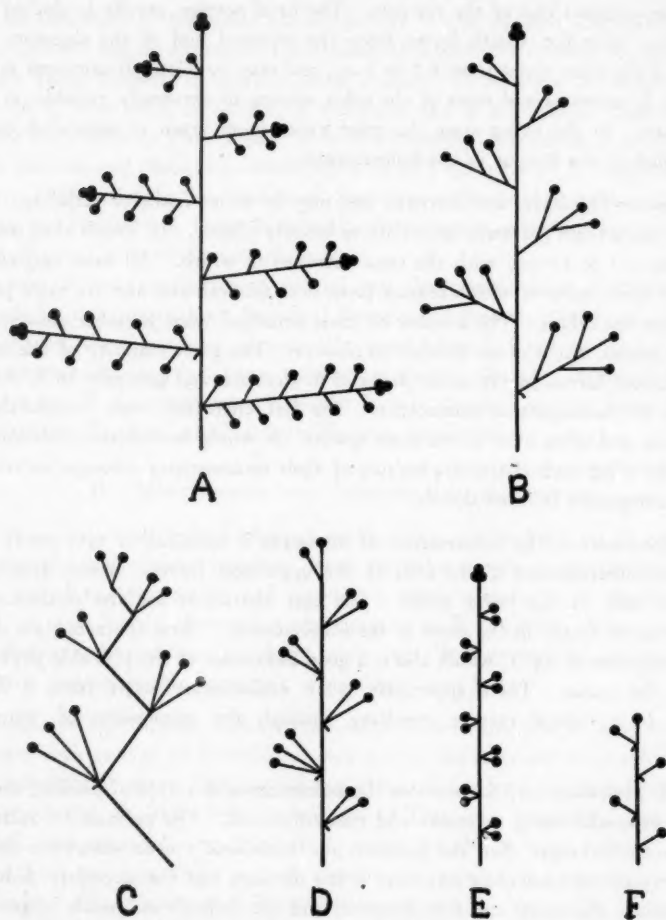


Figure 1. Inflorescence structures in *Smilacina*: A, *S. paniculata*; B, *S. amoena*, C, *S. flexuosa*; D, *S. scilloides*; E, *S. macrophylla*, F, *S. stellata*.

is subspiciform, with a straight, stout rachis bearing short, horizontal or reflexed, paired pedicels.

The reduction of the inflorescence to a typical raceme is attained in *S. stellata*, where the pedicels are borne singly at the nodes.

Perianth.—In *Smilacina*, as in most Liliaceae, there is no distinction of calyx

and corolla except in the separation of the six segments into an outer and an alternating inner whorl. The outer and inner segments are exactly alike in shape and size, varying amongst the different species from lanceolate to broadly ovate in outline, and in length from 1 mm. (*S. racemosa*) to 9 mm. (*S. flexuosa*). They may be widely spreading at anthesis, as in *S. paniculata*, or nearly erect and overlapping, as in *S. amoena* and *S. macrophylla*. The former condition is by far the more common. The perianth of *Smilacina* predominantly is white, but three closely related species, *S. flexuosa*, *S. scilloidea*, and *S. amoena*, include varieties with pink or flesh-colored flowers (*S. flexuosa* var. *rosea*, *S. scilloidea* var. *erubescens*, and *S. amoena* var. *Salvini*). This parallelism in color variation is very suggestive of close relationship amongst the three species.

Stamens.—There are six stamens in flowers of *Smilacina*, and these are attached to the base of the perianth segments. The anthers are introrse, 4-celled, and split longitudinally at anthesis. They vary from 0.5 to 3.0 mm. in length, and are commonly yellowish, but sometimes blue, at least in *S. paniculata*. The filaments may be narrowly filiform or somewhat enlarged at the base, varying from about 1 mm. in length in *S. racemosa* to 4 mm. in *S. flexuosa*.

Pistil.—The pistil is typically liliaceous, being 3-carpellate and 3-celled. The style may be somewhat shorter or somewhat longer than the ovary, and is capped by the stigma which is more or less deeply 3-lobed. *Smilacina* previously has been described as having two ovules within each loculus of the ovary, but during this investigation it has been found that the number varies from 1, in some flowers of *S. flexuosa* and *S. paniculata*, to 5 or 6 in *S. amoena*. Although the number of ovules is not a constant character for the species, there is a general tendency toward a constant number in each species.

Fruit.—The fruit is a one- to several-seeded berry, more or less deeply 3-lobed. It is some shade of red or dark purple at maturity, rather spotted when young.

The closest relatives of *Smilacina* in North America are the genera *Convallaria* L., *Maianthemum* Desf., *Clintonia* Raf., and *Polygonatum* Desf. These may be distinguished as follows:

- | | |
|--|-------------------|
| a. Perianth segments free. | |
| b. Stems leafy; leaves cauline, alternate; inflorescence paniculate to racemose. | |
| c. Flowers hexamerous | SMILACINA Desf. |
| cc. Flowers tetramerous | MAIANTHEMUM Desf. |
| bb. Stems scapiform; leaves basal; inflorescence umbellate | CLINTONIA Raf. |
| aa. Perianth segments coherent. | |
| b. Stems leafy; cauline leaves alternate; inflorescence axillary | POLYGONATUM Desf. |
| bb. Stems scapiform; leaves basal; inflorescence terminal | CONVALLARIA L. |

III. GEOGRAPHIC DISTRIBUTION

Smilacina is an outstanding example of the numerous genera having a common distribution in eastern Asia and eastern North America, to which Asa Gray called attention many years ago. In Asia the genus extends from Siberia to Japan and the Himalaya Mountains, represented by perhaps a dozen species. In North America its distribution ranges from Newfoundland to Alaska, southward to Panama.

In Mexico and Central America the genus is found only in the mountains at elevations of 1300-3300 m., usually in association with *Quercus*, *Alnus*, and other northern plant types. In such associations the plants of *Smilacina* frequently are common and terrestrial, but also grow on old stumps and mossy tree trunks from seeds probably deposited by birds after eating the pulpy berries.

Smilacina racemosa and *S. stellata*, which occur widely in the United States and northward, are found in Mexico only in the Sierra Madre of Chihuahua. But the majority of species have their center of distribution in Guatemala, extending northward to the Mexican States of Vera Cruz and Puebla and southward to the Province of Chiriquí, Panama. This distribution is that of *S. paniculata*, which is the most widespread of those in Mexico and Central America, as well as probably the most primitive of the species under discussion.

Smilacina amoena and *S. flexuosa* are nearly as widely distributed as *S. paniculata*, and occur throughout about the same territory from southeastern Mexico to Costa Rica. *Smilacina scilloidea* has been collected in Mexico, Honduras, and Guatemala, whilst *S. macrophylla* apparently is confined to southern Mexico. *Smilacina* apparently has not yet been found in Nicaragua, and only one doubtful specimen has been collected in Honduras. Future collections in those countries will surely show it to occur in the higher mountains, and help to explain some of the unsolved problems of this investigation.

IV. TAXONOMY

Smilacina Desf. in Ann. Mus. Paris 9:51, t. 9. 1807; Endl. Gen. 1183. 1836-40; Benth. & Hook. Gen. Pl. 3:770. 1883; Hemsl. Biol. Centr.-Am. Bot. 3:367. 1884; Engl. in Engl. & Prantl, Nat. Pflanzenfam. ed. 1. 2^d:79. 1888, *nomen conservandum*.

Vagnera Adans. Fam. Pl. 2:496. 1763, *nomen rejiciendum*.

Tovaria Neck. Elem. 2:190. 1790; J. G. Baker in Jour. Linn. Soc. Bot. 14:564. 1876;

Krause in Engl. & Prantl, Nat. Pflanzenfam. ed. 2. 15^a:367. 1930, *nomen rejiciendum*.

Polygonastrum Moench, Meth. 637. 1794, *nomen rejiciendum*.

Sigillaria Raf. in Jour. Phys. 89:261. 1819.

Stylandra Raf. loc. cit. 102. 1819.

Asteranthemum Kunth, Enum. Pl. 5:151. 1850.

Jocaste Kunth, loc. cit. 154. 1850.

Medora Kunth, loc. cit. 155. 1850.

Neolexis Salisb. Gen. Pl. Fragm. 64. 1866.

Perianth homochlamydeous, equally 6-parted, white to pink or purplish. Stamens 6, epipetalous, the filaments filiform or dilated, the anthers introrse, 4-celled. Ovary superior, 3-carpellate, the loculi with 1-6 superposed anatropous ovules on an axile placenta, the style terminal, filiform, the stigma capitate or somewhat 3-lobed. Berries pulpy, usually red or purplish, 1-6-seeded. Herbs with simple stems from an extensive rhizome. Leaves alternate, shortly petiolate to sessile or somewhat amplexicaul, with 3 to many principal parallel veins and variable secondary and cross veins. Inflorescence usually terminal, occasionally in the upper leaf axils also, paniculate to racemose, bracts very small or lacking.

Type species: *Smilacina racemosa* (L.) Desf.

Specimens have been examined from five of the larger herbaria of the United States. These are abbreviated in the citation of exsiccatæ as follows: Chicago Museum of Natural History (formerly Field Museum) (FM); Gray Herbarium of Harvard University, Cambridge, Mass. (GH); Missouri Botanical Garden, St. Louis (MBG); University of California, Berkeley (UC); United States National Herbarium, Washington (US). I am very grateful to the curators of these herbaria for their courtesy and interest, and particularly to Dr. J. M. Greenman, Curator of the Missouri Botanical Garden Herbarium, under whom this investigation was carried on.

KEY TO THE SPECIES AND VARIETIES

- a. Inflorescence paniculate, the lateral branches of the rachis manifest (but the upper ones much reduced in *S. amoena*).
 - b. Inflorescence typically paniculate, the lateral branches of the rachis many-flowered; pedicels scarcely longer than the perianth.
 - c. Perianth segments 3-5 mm. long; anthers included..... 1. *S. paniculata*
 - cc. Perianth segments 1.0-1.5 mm. long; anthers exerted..... 2. *S. racemosa*
 - bb. Inflorescence subracemiform, the lateral branches of the rachis corymbose, less manifest and few-flowered, the upper ones much reduced; pedicels much longer than the perianth.
 - c. Flowers white..... 3. *S. amoena*
 - cc. Flowers pink..... 3a. *S. amoena*
var. *Salvini*
- aa. Inflorescence subracemose, the lateral branches of the rachis not manifest, or only rarely so, the flowers paired or clustered at the nodes.
 - b. Inflorescence racemiform, the rachis scarcely stouter than the pedicels, the latter usually horizontal or ascending; perianth segments spreading, oblong-lanceolate, 2-3 times as long as broad.
 - c. Rachis flexuous or geniculate; pedicels longer than the perianth; principal veins of leaves usually 5-7.
 - d. Flowers white..... 4. *S. flexuosa*
 - dd. Flowers purplish pink..... 4a. *S. flexuosa*
var. *erubescens*
 - cc. Rachis straight; pedicels as long as the perianth or somewhat shorter; principal veins of leaves usually 3.
 - d. Flowers white..... 5. *S. scilloidea*
 - dd. Flowers pink..... 5a. *S. scilloidea*
var. *rosea*
 - bb. Inflorescence subspiciform, the rachis straight, much stouter than the pedicels, the latter descending; perianth segments nearly erect, broadly oval, almost as broad as long..... 6. *S. macrophylla*
 - aaa. Inflorescence typically racemose, the rachis unbranched, the flowers solitary in the axils of minute bracts..... 7. *S. stellata*

1. *Smilacina paniculata* Mart. & Gal. in Bull. Acad. Brux. 9²:388. 1842.

Tovaria thyrsoides J. G. Baker in Jour. Linn. Soc. Bot. 14:568. 1876.

Tovaria laxiflora Baker, loc. cit. 569. 1876.

Tovaria nervulosa Baker, loc. cit. 1876.

Smilacina thyrsoides (Baker) Hemsl. Biol. Centr.-Am. Bot. 3:368. 1884.

Smilacina laxiflora (Baker) Hemsl. loc. cit. 1884.

Smilacina nervulosa (Baker) Hemsl. loc. cit. 1884.

Smilacina Gigas Woodson, Ann. Mo. Bot. Gard. 27:270. 1940.

Stems 0.4–3 m. high, somewhat flexuous, glabrous; leaves shortly petiolate, narrowly lanceolate to broadly ovate, acuminate, 6–30 cm. long, 1–12 cm. broad, longitudinal veins closely parallel and equal, or widely separated with 3–8 prominent veins and less conspicuous intermediate parallel veins, lateral veins more or less visible in dried plants; inflorescence typically paniculate, the secondary branches extensive and many-flowered, 3–50 cm. long, 2–25 cm. broad; pedicels solitary, horizontal or ascending, 1–10 mm. long; flowers white; perianth segments ovate-lanceolate, 3–5 mm. long, 1.5–2.5 mm. broad, spreading; stamens included, 2–4 mm. long, filaments somewhat enlarged at base, 1–3 mm. long, anthers 0.5–2.5 mm. long; ovary and style about equal in length, 1–5 ovules in each loculus, 1–2 ovules most common; fruit 1–5-seeded.

MEXICO: VERA CRUZ: Orizaba, *Botteri* 138 (GH, US); in wet forest, in region of Orizaba, *Botteri* 914 (US); in moist mountain forest, Cerro de Chocoman, Canton Córdoba, May 12, 1907, *Seler & Seler* 5174 (US, GH); Acultzingo, May 1, 1937, *Matuda* 1161 (US). PUEBLA: ledges of barranco below Honey Station, alt. 1525 m., May 6, 1904, *Pringle* 8836 (UC, FAL, MBG, US). OAXACA: Teotcalcingo, Petlapa, alt. 800–1400 m., *Galeotti* 5485 (GH, UC, US); Petlapa, June, *Liebmann* 14637 (FM). CHIAPAS: Cerro del Boquerón, Aug. 1913, *Purpus* 7022 (UC).

GUATEMALA: ALTA VERAPAZ: on stumps and banks, Chama to Cobán, alt. 1075 m., Aug. 23, 1920, *Johnson* 639 (US); forest near Cobán, alt. 1600 m., Sept. 1907, *Tuerckheim* II 1962 (GH, US); Pansamalá, alt. 1125 m., May 1887, *Tuerckheim* 231 (US). SAN MARCOS: hanging from tree at upper edge of potrero, Volcán Tajumulco, alt. 1300–1350 m., March 13, 1940, *Steyermark* 37655 (FM). BAJA VERAPAZ: damp forest, mountain side north of divide north of Santa Rosa, alt. 1650 m., March 30, 1939, *Standley* 69927 (FM).

COSTA RICA: CARTAGO: Volcán Irazú, alt. 3050–3450 m., Dec. 1, 1937–Jan. 1, 1938, *Allen* 690 (FM); in the oak forest on the upper slopes, El Volcán Irazú, Aug. 18, 1925, *Dodge* 3417 (GH, US); south slope of Volcán Irazú near Finca Chilena, alt. 2700–2900 m., March 4, 1930, *Dodge & Thomas* 8073 (GH, MBG); southern slope of western or main cone of Irazú, above alt. 3050 m., July 31, 1937, *Hatch* 178 (FM); vicinity of the crater of the Volcán Irazú, Aug. 24, 1935, *Quiros* 336 (FM); southern slope of Volcán de Irazú, March, 1924, *Standley* 36629 (US); southern slope of Volcán de Turrialba, near the Finca del Volcán de Turrialba, alt. 2000–2400 m., Feb. 22, 1924, *Standley* 35186 (US). SAN JOSÉ: on tree in wet forest, near Finca la Cima, above Los Lotes, north of El Copey, alt. 2100–2400 m., Dec. 21–22, 1925, *Standley* 42735 (US); La Hondura, alt. 1300 m., Aug. 15, 1933, *Valerio* 790 (FM).

PANAMA: CHIRIQUÍ: vicinity of "New Switzerland", central valley of Río Chiriquí Viejo, alt. 1800–2000 m., Jan. 6–14, 1939, *Allen* 1392 (MBG); trail from Cerro Punta to headwaters of Río Caldera, alt. 2250–2500 m., Jan. 14, 1939, *Allen* 1446 (MBG); rain forest, Bajo Chorro, alt. 1825 m., Jan. 6, 1938, *Davidson* 53 (FM); Volcán de Chiriquí, Boquete District, alt. 2900 m., July 16, 1938, *Davidson* 990 (FM); Volcán de Chiriquí, alt. 3300 m., Feb. 27, 1918, *Killip* 360 (US); forest edge, vicinity El Potrero Camp, Chiriquí Volcano, alt. 2800–3000 m., March 10–13, 1911, *Pittier* 3071 (US); on rotten stumps, Valley of the upper Río Chiriquí Viejo, vicinity of Monte Lirio, alt. 1300–

1900 m., June 27–July 13, 1935, *Seibert 184* (GH, MBG); vicinity of Casita Alta, Volcán de Chiriquí, alt. 1500–2000 m., June 28–July 2, 1938, *Woodson, Allen & Seibert 852* (MBG); Loma Larga to summit, Volcán de Chiriquí, alt. 2500–3380 m., July 4–6, 1938, *Woodson, Allen & Seibert 1034* (MBG); valley of the upper Río Chiriquí Viejo, March 18, 1938, *P. White 57a* (MBG); valley of the upper Río Chiriquí Viejo, Summer 1937, *P. White 75* (MBG); vicinity of Bajo Mona and Quebrada Chiquero, alt. 1500 m., July 18, 1940, *Woodson & Schery 512* (MBG); Casita Alta to Cerro Copete, alt. 2300–3300 m., July 10, 1940, *Woodson & Schery 339, 341, 342* (MBG).

This species is extremely variable in size, ranging from 0.4 to 3.0 m. tall. The leaves are proportionately variable also. The inflorescence may be small, measuring only 3 cm. long and 2 cm. broad, or it may be large and spreading, 50 cm. long and 25 cm. broad.

2. *Smilacina racemosa* (L.) Desf. in Ann. Mus. Par. 9:51. 1807.

Convallaria racemosa L. Sp. Pl. 315. 1753.

Tovaria racemosa Neck. Elem. 3:190. 1790.

Polygonastrum racemosum Moench, Meth. 637. 1794.

Maianthemum racemosum Link, Enum. 1:343. 1821.

Unifolium racemosum Britton in Trans. N. Y. Acad. Sci. 8:74. 1889.

Vagnera racemosa Morong in Bull. Torr. Bot. Club 20:480. 1893.

Stems 1.8–7.8 dm. high, flexuous, glabrous or slightly pubescent; leaves sessile or shortly petiolate, lanceolate-ovate, acuminate, under leaf-surface inconspicuously pubescent, chiefly lower portion and petiole, 3–4 times as long as broad, 6–15 cm. long, 1.5–5.5 cm. broad, many major and minor longitudinally parallel veins, lateral veins hidden; inflorescence typically paniculate, lateral branches several-flowered, 2.5–10.5 cm. long, 0.5–2.5 cm. broad; pedicels solitary, usually ascending or horizontal, 0.5–2 mm. long; flowers white; perianth segments lanceolate or oblong, 1.0–1.5 mm. long, 0.5 mm. broad; stamens exerted, 2–3 mm. long, filaments enlarged at the base, 1.5–2.5 mm. long, anthers 0.5 mm. long; style shorter than the ovary, 2 ovules in each loculus; fruit 1–several-seeded.

MEXICO: CHIHUAHUA: near Colonia Garcia in the Sierra Madres, alt. 2285 m., June 3, 1899, *Townsend & Barber 5* (FM, MBG, US); Las Cuevas, June 30, 1892, *Hartmann 544* (GH, US) cold ledges, Sierra Madre, Oct. 3, 1887, *Pringle 1487* (GH); Sierra Madre, 1899, *Townsend & Barber 8* (US).

With so few specimens, it appears unprofitable to attempt the association of our Central American *S. racemosa* with either var. *typica* or var. *cylindrata* as segregated by Fernald (in *Rhodora* 40:406–407. 1938). Amongst even our four specimens, extent of branching and general shape of the inflorescence are quite variable, suggesting the hopeless variability in *S. paniculata*. Evaluation of the reported geographical gradient in *S. racemosa* of the United States is without the scope of this discussion.

3. *Smilacina amoena* Wendl. in Otto & Dietr. Allg. Gart. Zeit. 17:137. 1850.

Tovaria paniculata Baker, loc. cit. 568. 1876, as to description and specimens cited, not *Smilacina paniculata* Mart. & Gal.

Smilacina paniculata Mart. & Gal. acc. to Hemsl. Biol. Centr.-Am. Bot. 3:368. 1884, excluding specimens cited.

Stems 1.5–8.0 dm. high, somewhat flexuous, glabrous; leaves sessile and amplexicaul or shortly petiolate, narrowly ovate to broadly ovate-acuminate, 5–20 cm. long, 2–8 cm. broad, glabrous, 4–12 prominent longitudinal veins, with numerous and less conspicuous parallel veins between, lateral veins visible in dried plants; inflorescence subracemiform, not flexuous, 5–30 cm. long, 1.5–10 cm. broad; secondary branches much reduced above, few-flowered; pedicels 4–30 mm. long, horizontal or ascending; flowers white; perianth segments narrowly elliptic-obovate, 5–6 mm. long, 2.5–3.0 mm. broad, somewhat spreading; stamens included, 4 mm. long, filaments less than 1 mm. broad at the base, 3 mm. long, anthers 1.0–1.5 mm. long; ovary and style about equal in length, 4–6 ovules in each loculus; mature fruit little known.

MEXICO: VERA CRUZ: Nogales, May 2, 1937, *Matuda 1154* (MBG, US). CHIAPAS: 1864–1870, *Gbiesbreght 708* (GH, MBG).

GUATEMALA: ZACAPA: cloud forest in ravine bordering Quebrada Alejandria, summit of Sierra de las Minas, vicinity of Finca Alejandria, alt. 2500 m., Oct. 13, 1939, *Steyermark 29875* (FM). CHIMALTENANGO: Calderas, Oct. 25, 1937, *Johnson 1109* (FM).

COSTA RICA: SAN JOSÉ: on tree in wet forest, near Finca la Cima, above Los Lotes, north of El Copey, alt. 2100–2400 m., Dec. 21–22, 1925, *Standley 43587* (US); high plateau, alt. 2000 m., Jan. 23, 1935, *Valerio 1075* (FM). ALAJUELA: San Ramón, April 21, 1929, *Brenes 6828* (FM).

3a. *Smilacina amoena* var. *Salvini* (Baker) Emons, n. comb.

Tovaria Salvini J. G. Baker in Jour. Linn. Soc. Bot. 14:567. 1876.

Smilacina Salvini (Baker) Hemsl. Biol. Centr.-Am. Bot. 3:368. 1884.

Essentially the same as *S. amoena* but with flowers pink; perianth segments broadly elliptic, 6 mm. long, 3.5–4.5 mm. broad, erect.

GUATEMALA: CHIMALTENANGO: Bosques de la Sierra "Santa Elena", Tecpam, alt. 2500 m., Jan. 1, 1932, *Salas 1430* (FM); Tecpam, alt. 2740 m., Feb. 5, 1937, *Johnson 633* (FM); Santa Elena, 1933, *Skutch 232* (US); place not recorded, Jan. 1892, *Shannon 439* (US); Chicoy, alt. 2500 m., March 1892, *Shannon 358* (US); Volcán Zunil, alt. 2500–3800 m., Jan. 22, 1940, *Steyermark 34685* (FM); above Tecpam, March 11, 1931, *Collins & Kempton 21* (US); Cupressus forest, Cerro de Tecpam, region of Santa Elena, alt. 2400–2700 m., Dec. 26, 1938, *Standley 61123* (FM). QUEZALTENANGO: Volcano of Santa Maria, alt. 2400–3510 m., Jan. 24, 1896, *Nelson 3700* (GH, US). SAN MARCOS: along Quebrada Canjula, between Sabinal and Canjula, Volcán Tacana, alt. 2200–2500 m., Feb. 18, 1940, *Steyermark 36024* (FM); between San Sebastián and top of ridge of Volcán Tajumulco, alt. 3800–4000 m., Feb. 16, 1940, *Steyermark 35903* (FM). HUEHUETENANGO: on mountain between Sija and Huehuetenango, alt. 3000 m., Feb. 21, 1938, *Walsh s. n.* (MBG).

4. *Smilacina flexuosa* Bertol. in Nov. Comm. Acad. Bonon. 4:411. pl. 39. 1840; Hemsl. Biol. Centr.-Am. Bot. 3:367. 1884.

Smilacina Bertolonii Kunth, Enum. 5:151. 1850.

Tovaria flexuosa (Bertol.) Baker in Jour. Linn. Soc. Bot. 14:567. 1876.

Convallaria flexuosa Druce, Rept. Bot. Exch. Club Brit. Isl. 3:408. 1914.

Vagnera flexuosa Standl. in Jour. Wash. Acad. Sci. 15:457. 1925.

Stems 0.3–9 dm. high, more or less straight, glabrous or pubescent; leaves subsessile to shortly petiolate, narrowly lanceolate to broadly ovate and shortly acuminate, 6–22 cm. long, 1.2–7 cm. broad, 5–7 prominent longitudinal veins,

intermediate ones less conspicuous, numerous short lateral veins; inflorescence racemiform, 5–30 cm. long, 2–6 cm. broad; rachis geniculate or flexuous; pedicels paired or clustered at the nodes, 0.7–3 cm. long, scarcely thinner than the rachis, horizontal or ascending; flowers white; perianth segments oblong-lanceolate, 5–9 mm. long, 2–2.5 mm. broad, spreading; stamens included, 3–4 mm. long, filaments slightly enlarged at the base, 2–3 mm. long, anthers about 1 mm. long; style somewhat longer than the ovary, stigma slightly lobed, 1–2 ovules in each loculus, usually 1; fruit usually 1–4-seeded.

MEXICO: CHIAPAS: Cerro del Boquerón, Aug., 1913, *Purpus* 7022 (US); damp forests, mountains east of Fenix or Phoenix, date lacking, *Purpus* 10621 (US); Cerro del Boquerón, June, 1914, *Purpus* 7416 (FM, UC, US); Chicharras, alt. 3000–6000 ft., Feb. 6, 1896, Nelson 3762 (US).

GUATEMALA: ALTA VERAPAZ: near Tactic, alt. about 1500 m., April 5, 1939, *Standley* 70485 (FM); San Martín, July 1, 1938, *Johnston* 1305 (FM). BAJA VERAPAZ: Tactic, alt. 1400 m., April, 1882, *Lehmann* 1315 (US). HUEHUETENANGO: Concepción bei San Martín im Gebüsch, alt. 2000 m., June 21, 1896, *Seler & Seler* 3168 (GH, US). SAN MARCOS: shaded moist ravine slopes, between San Rafael and Guatemala-Mexico line, alt. 2500–3000 m., Feb. 21, 1940, *Steyermark* 36321 (FM); barrancos 6 miles south and west of town of Tajumulco, alt. 2300–2800 m., Feb. 26, 1940, *Steyermark* 36618 (FM); along road above Barranco Eminencia, alt. about 2700 m., March 14, 1939, *Standley* 68568 (FM). JALAPA: Volcán Jumay, north of Jalapa, alt. 1300–2200 m., Dec. 1, 1939, *Steyermark* 32400 (FM). CHIMALTENANGO: Chichavac, alt. 2400–2700 m., Nov.-Dec., 1930, *Skutch* 73 (US); Cerro de Tecpam, region of Santa Elena, alt. about 2700 m., Dec. 4, 1938, *Standley* 58708 (FM); region of Las Calderas, alt. 1800–2100 m., Nov. 22, 1938, *Standley* 57810 (FM). SACATEPEQUEZ: slopes of Volcán de Agua, above Santa María de Jesús, alt. 2250–3000 m., Feb. 11, 1939, *Standley* 65134 (FM); Volcán Agua, alt. 9500 ft., Feb. 8, 1908, *Kellerman* 7295 (FM). SANTA ROSA: Santa Rosa, alt. 840 m., June, 1892, *Heyde & Lux* 3527 (US); Volcán de Agua, alt. 2800 m., June, 1892, *Shannon* 3634 (US).

EL SALVADOR: Cerro de Apaneca, 1928, *Calderon* 2417 (FM).

HONDURAS: in forest near summit of the range above El Achote, in cloud zone above the plains of Siguatepeque, alt. 1850 m., Aug. 1, 1936, *Yuncker, Dawson & Youse* 6267 (FM).

COSTA RICA: CARTAGO: El Muneco, alt. 5000 ft., June 19, 1928, *Stork* 2714 (FM).

4a. *Smilacina flexuosa* Bertol. var. *erubescens* Emons, n. var.¹⁸

Flowers pink; otherwise essentially the same as the species.

MEXICO: CHIAPAS: Cerro del Boquerón, June, 1914, *Purpus* 7415 (FM, GH, UC); Volcán Tacana, Chiquihuite, March 27, 1939, *Matuda* 2846 (FM).

GUATEMALA: GUATEMALA: locality lacking, 1939, *Aguilar* 232 (FM). ZACATEPEQUEZ: Volcán de Agua, alt. 2800 m., J. D. Smith 2175 (US). QUICHE: Nebaj, alt. 1930 m., April, 1890, *Heyde & Lux* 4647 (GH, US). SANTA ROSA: Zamorora, alt. 1535 m., April, 1893, *Heyde & Lux* 4652 (GH, US). SAN MARCOS: between Todos Santos and Finca El Porvenir, alt. 1300–3000 m., March 1, 1940, *Steyermark* 36972 (FM, TYPE); San Martín, Oct. 9, 1938, *Johnston* 1305 (FM).

5. *Smilacina scilloidea* Mart. & Gal. in Bull. Acad. Brux. 9:388. 1842.

Smilacina scilloidea var. *acutifolia* Mart. & Gal. loc. cit. 1842.

Tovaria scilloidea [*scilloides*] (Mart. & Gal.) Baker in Jour. Linn. Soc. Bot. 14:567. 1875 (?), misspelling.

¹⁸ *Smilacina flexuosa* Bertol. var. *erubescens* Emons, var. nov., ab specie floribus roseis praecipue differt.

Stems 1.5–8 cm. high, flexuous, glabrous; leaves slightly sessile to petiolate, narrowly lanceolate to ovate, shortly acuminate, 3.5–10 cm. long, 1.0–4.5 cm. broad, usually with about 3 major longitudinal veins and numerous minor ones between, lateral veins evident in dried plants; inflorescence racemiform, 4–6 cm. long, 1.3–2.5 cm. broad; rachis straight, pedicels usually paired at the nodes, 3–6 mm. long, scarcely thinner than the rachis, horizontal or ascending; flowers white; perianth-segments oblong-lanceolate, usually 4–5 mm. long, rarely 6 mm., 1–2 mm. broad, spreading; stamens included, about 3 mm. long, filaments 1.5–2 mm. long, anthers 1 mm. or less long; style and ovary usually about equal length, style sometimes longer, stigma slightly 3-lobed, 1–2 ovules in each loculus, commonly 2; mature fruit several-seeded.

MEXICO: MICHOACÁN: vicinity of Morelia, Cerro Azul, alt. 2200 m., 1910, *Arsène* 5766 (MBG, GH); vicinity of Morelia, Companario, alt. 2100 m., Dec. 1910, *Arsène* 5803 (US). OAXACA: 2230–2500 m., *Galeotti* 5483 (US); Cerro San Felipe, alt. 3000 m., May 22, 1898, *Conzatti & Gonzalez* 704 (GH, US); rich canyons, Sierra de San Felipe, alt. 2230–2740 m., May 22, 1894, *Pringle* 4647 (GH, MBG, UC, US); northwest slope of Mt. Zempoaltepec, alt. 2230–3045 m., July 10, 1894, *Nelson* 667 (US); San Miguel, alt. 3000 m., May 1917, *Reko* 3840 (US); Lachopa, June 1841, *Li-bmann* 14630 (FM). CHIAPAS: 1864–1870, *Gbiesbreght* 707 (GH, MBG); Cerro del Boquerón, June 1914, *Purpus* 7416 (GH, MBG); District of Temascaltepec, Nanchititla, Aug. 12, 1933, *Hinton* 4520 (FM, MBG, US); Cajones, July 9, 1935, *Hinton* 7953 (MBG, US); oak woods, Temascaltepec, Nov. 8, 1933, *Hinton* 5078 (FM).

HONDURAS: COMAYAGUA: in the forest near the summit of the range above El Achote, in cloud zone above the plains of Siguatepeque, alt. 1850 m., Aug. 1, 1936, *Yuncker, Dawson & Youse* 6257 (FM, MBG).

5a. *Smilacina scilloidea* Mart. & Gal. var. *rosea* Emons, n. var.¹⁹

Flowers pink; otherwise essentially the same as the species.

GUATEMALA: CHIMALTENANGO: in open pine forest with dense tussock grass, slopes of Volcán de Acatenango, above Las Calderas, alt. 2700–2900 m., Jan. 3, 1939, *Standley* 61890 (FM). QUEZALTENANGO: pine-fir forest, Volcán Zunil, alt. 2500–3800 m., Jan. 22, 1940, *Steyermark* 34720 (FM); Volcán Santa María, alt. 3260 m., July 27, 1934, *Skutch* 869 (FM, TYPE, GH).

6. *Smilacina macrophylla* Mart. & Gal. in Bull. Acad. Brux. 9²:387. 1842.

Stems 4.5–6.0 dm. high, somewhat flexuous, glabrous; leaves shortly petiolate or subsessile, ovate-lanceolate, acuminate, 12.5–22.0 cm. long, 2.5–9.0 cm. broad, with 5–10 prominent longitudinal veins and less conspicuous ones between, lateral veins visible in dried plants; inflorescence subspiciform, several-flowered, 12.5–22.0 cm. long, 1.2–2.0 cm. broad; peduncle straight, much stouter than the pedicels; pedicels solitary, paired or clustered, 3–5 mm. long, horizontal or descending; flowers white (?); perianth segments broadly oval, 6 mm. long, 3.0–3.5 mm. broad, nearly erect; stamens included, filaments enlarged at base, 3 mm. long, anthers 1.5 mm. long; ovary and style of equal length, 4 ovules to each loculus; mature fruit unknown.

¹⁹ *Smilacina scilloidea* Mart. & Gal. var. *rosea* Emons, var. nov., ab specie floribus roseis praecipue differt.

MEXICO: VERA CRUZ: barranco of Teoxolo, near Jalapa, alt. 3500 ft., May 22, 1899, Pringle 7854 (GH, US); Huitamalco, June 1841, Liebmman 14635 (US, FM).

I have not seen the type specimen of *S. macrophylla*, Galeotti 5473, but the plants cited above agree well with the original description.

7. *Smilacina stellata* (L.) Desf. in Ann. Mus. Par. 9:52. 1807.

Convallaria stellata L. Sp. Pl. 316. 1753.

Tovaria stellata (L.) Neck. Elem. 3:190. 1790, name only.

Maianthemum stellatum (L.) Link, Enum. 1:343. 1821.

Asteranthemum vulgare Kunth, Enum. 5:152. 1850.

Unifolium stellatum (L.) Greene in Bull. Torr. Bot. Club 15:287. 1888.

Vagnera stellata (L.) Morong in Mem. Torr. Bot. Club 5:114. 1894.

Stems 1.5–5.5 dm. high, somewhat flexuous, glabrous; leaves sessile, amplexicaul, narrowly elliptical-lanceolate, acuminate, 4–15 cm. long, 1.0–4.5 cm. broad, upper leaf-surface glabrous, lower surface pubescent, many parallel longitudinal veins more or less of equal prominence, lateral veins usually hidden; inflorescence typically racemose, 2.5–7.0 cm. long, 1.5–2.0 cm. broad; peduncles unbranched, more or less straight; pedicels solitary, 2–10 mm. long, ascending; flowers white; perianth segments narrowly elliptical-oblong, 5–7 mm. long, 1.5–2.5 mm. broad, spreading; stamens included, 4 mm. long, filaments somewhat enlarged at base, 3 mm. long, anthers 1 mm. or less long; ovary usually longer than style, 2 ovules to each loculus; mature fruit 1–2-seeded.

MEXICO: CHIHUAHUA: collected near Colonia Garcia in the Sierra Madres, alt. 2290 m., June 16, 1899, Townsend & Barber 35 (FM, GH, MBG, UC, US). NUEVO LEÓN: in shelter of thickets below timberline, alt. 11,700 ft., Cerro Potosi, July 9, 1938, Schneider 1033 (FM).

This species is chiefly one of the United States, and reaches only a limited portion of northern Mexico where it is found in the higher altitudes. It is easily distinguished by its pubescent, sessile leaves, and simple raceme.

EXPLANATION OF PLATE

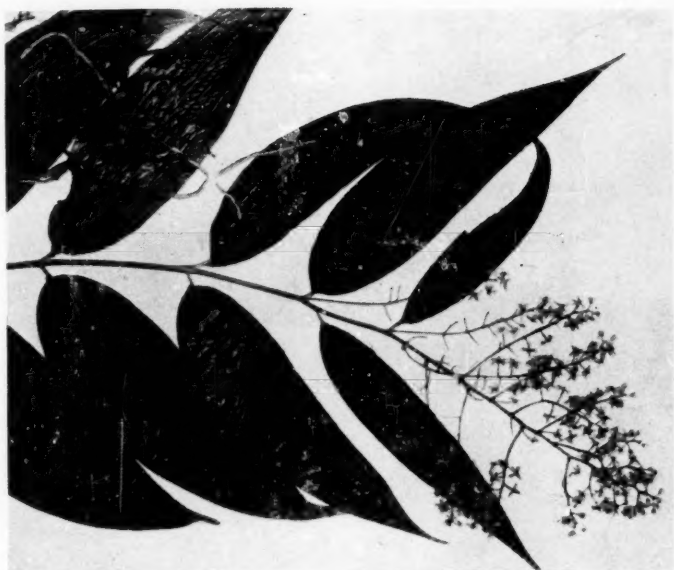
PLATE 18

Fig. 1. *Smilacina paniculata* Mart. & Gal. Specimen collected in Valley of the Upper Río Chiriquí Viejo, vicinity of Monte Lirio, Province of Chiriquí, Panama, R. J. Seibert 184, in the Herbarium of the Missouri Botanical Garden.

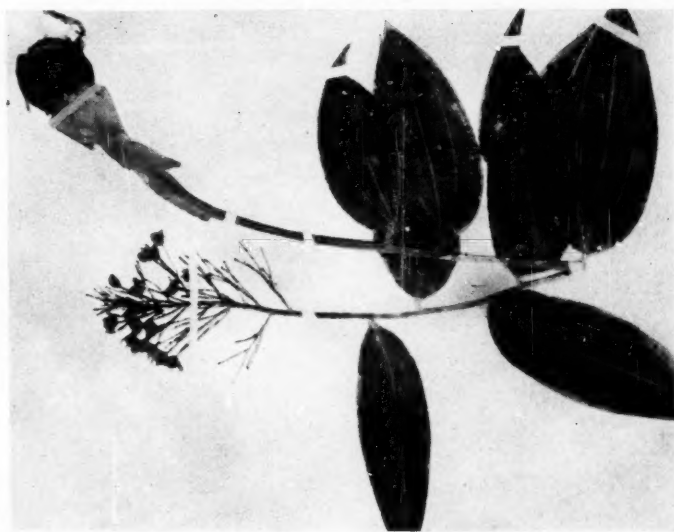
Fig. 2. *Smilacina amoena* Wendl. var. *Salvini* (Baker) Emons. Epiphyte collected along Quebrada Canjula, between Sabinal and Canjula, Volcán Tacaná, alt. 2200–2500 m., Dept. San Marcos, Guatemala, J. A. Steyermark 36024, in the Herbarium of the Chicago (Field) Museum of Natural History.

1

EMONS—CENTRAL AMERICAN SMILACINAS



2



EXPLANATION OF PLATE

PLATE 19

Fig. 1. *Smilacina flexuosa* Bertol. Specimen collected in Chicharras, Chiapas, Mexico, E. W. Nelson 3762, in the United States National Herbarium.

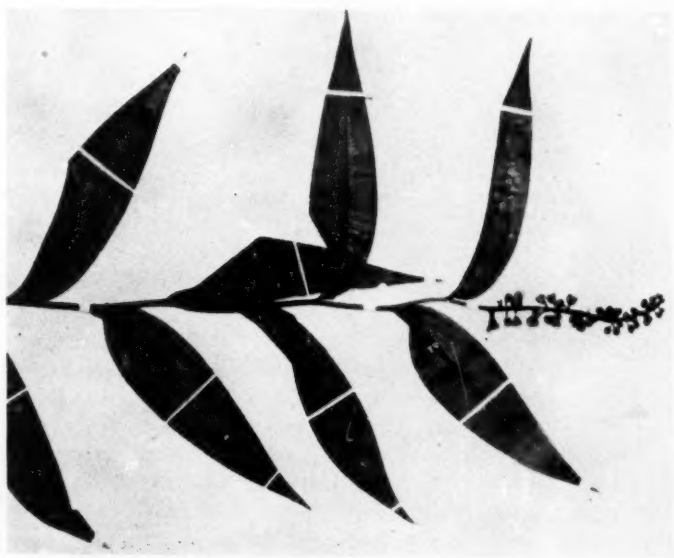
Fig. 2. *Smilacina macrophylla* Mart. & Gal. Specimen collected in barrancos of Teoxolo, Vera Cruz, Mexico, C. G. Pringle 7854, in the United States National Herbarium.

1



EMONS—CENTRAL AMERICAN SMILACINAS

2



THE CLEMATIS FREMONTII VAR. RIEHLII POPULATION IN THE OZARKS¹

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INTRODUCTION

During the latter half of the nineteenth century, the Darwinian theory of evolution by natural selection inspired a vast amount of research which was largely directed toward tracing of phylogenies and demonstrating the adaptation of organisms to their environment. However, the theory has recently been somewhat out of fashion. Its abeyance was coincident with the rise after 1900 of the new science of genetics and its companion, modern nuclear cytology. Preoccupation with the new disciplines partly accounted for the neglect of evolutionary studies. But it was partly due to the fact that the new principles which were emerging, the particulate theory of inheritance and the DeVriesian mutation theory, seemed to contradict some of the premises of Darwinism.

To-day there is a resurgence of interest in evolutionary matters. It is apparent that Darwin's theory, in its essentials, still stands. Modern genetics throws immediate light on some points which were hidden to Darwin. Gene mutation, which has now been studied in the laboratory and in the field, is seen to be the source of the omnipresent variation which Darwin pointed out but did not explain. The particulate nature of inheritance, far from being contradictory to the theory of natural selection, has been shown by Fisher ('30) to be essential to evolutionary change. A mathematical theory has been constructed, largely by Wright (for bibliography and non-mathematical summary, see Dobzhansky, '41), which permits rates of change of gene frequency to be calculated from mutation rates, the selective advantage of one gene over another, and size of population, under various systems of mating. These changes in gene frequency, when integrated for the entire genotype of the organism and over its entire population, may be said to constitute the primary steps in evolution.

The most important generalizations which Wright has made from his mathematical studies are those relating to the effect of population size, or more accurately, of what he terms "population number," upon the rate and course of evolution. In a very large, freely interbreeding population, where the number of potential mates for each breeding individual is large in relation to mutation rates, selection is strongly operative. The genotypes of the organism will then tend to cluster closely about a peak in the surface of adaptive values. The organism will be well adapted to its environment, but its over-all variability will be somewhat

¹ This paper is a revision of a dissertation which was prepared in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

restricted. It will adapt itself to a secular change in the environment by moving to a new adaptive peak, but it will not be able to cross an adaptive valley to reach a conceivably higher peak. In a very small population, or in one which is divided into small isolated colonies, the range of variation will be restricted locally, though there may be considerable variation from one colony to another. The phenomenon of "genetic drift" will come into play. There will be a random loss and fixation of genes resulting from the errors of sampling of the gametes which reproduce each generation, largely without regard to the adaptive value of the genes involved. As a result, the fate of an organism which is too greatly restricted in numbers is extinction. Wright considers the most favorable condition for continuing evolution to be that of a large population broken up into numerous small colonies which are connected by occasional migration. Each colony will be free to explore the field of gene combinations without the restrictive effect of too rigid selection. Differentiation within the population will be largely non-adaptive, but some of the colonies will be expected to arrive at favorable genotypes or adaptive peaks, perhaps quite different from the original one about which the population centered. Such colonies will tend to increase in numbers and to bring the remainder of the population up to their genotype through migration. This combination of non-adaptive differentiation of partially isolated local groups with intergroup selection will permit evolutionary advance without a secular change in conditions.

Wright's theory has become an important part of modern evolutionary thought. Eventually it may have the same importance and validity in the field of evolution which the publications of J. Willard Gibbs have in chemical thermodynamics. However, it is merely a theory, and it is impossible at present to judge whether it adequately accounts for evolutionary changes which are known to take place. It urgently requires testing against facts from the field. The facts required for its examination, or the examination of any other theory which attempts to explain the mechanism of evolutionary change, are of many kinds. The beauty of Wright's theory is that it indicates clearly the kinds of information which are important. Detailed information is required about life histories of various organisms, particularly the details of reproduction. Data are required on the numbers of individuals, and on their pattern of distribution, both at present and over a span of years. The pattern of differentiation must be understood in detail. Detailed information about sources of evolutionary change such as mutation, hybridization, and chromosomal changes must be obtained. Furthermore, the data on all these points must be coordinated for individual organisms. Such a body of detailed and coordinated facts scarcely exists for any organism, but is of first importance in any discussion of evolution.

The present study of *Clematis Fremontii* var. *Rieblii* was undertaken with the object of working out a picture of the features of its population structure which are of evolutionary importance, and if possible of making an estimate of evolutionary trends within the population. The pattern of distribution has been worked

out in some detail. Biological factors such as method of pollination, seed dissemination, seed germination and longevity have been examined. Variation in flower and leaf characters has been studied. An attempt has been made to obtain quantitative data where possible, but many of the present conclusions are based on subjective judgment; the difficulties are many.

Clematis Fremontii var. *Rieblii* is a member of the section VIORNA, subsection INTEGRIFOLIAE of *Clematis* (Erickson, '43a). Besides the Eurasian *C. integrifolia*, which probably should be placed in the subsection, it includes four closely related species and one or two varieties. They are comparatively well-marked and uniform entities, contrasting with such polymorphic species as *C. Pitcheri*. All except *C. ochroleuca* are of restricted distribution, characteristically occurring on rocky barrens. *C. albicoma* and the recently proposed *C. albicoma* var. *coactilis* (Fernald, '43) occur on the Devonian shale barrens of the Appalachians of West Virginia and Virginia (Wherry, '30, '31). *C. viticaulis*, also a shale barren plant, has been collected at a single locality. *C. Fremontii* is a secondary species in the *Andropogon scoparius* habitat of the mixed prairie of north-central Kansas. There it is usually limited to the upper slopes above the brows of hills where there is an outcrop of Fort Hays Limestone or Smoky Hill Chalk (Albertson, '37, '42).

C. Fremontii var. *Rieblii* is restricted to an area of somewhat more than 400 sq. mi. in Jefferson County and portions of two adjacent counties in east-central Missouri. A distribution map and a discussion of the limits of its distribution have been published (Erickson, '43b). The plant is wholly restricted to glades, rocky barrens which occur on south- and west-facing slopes of otherwise wooded ridges. The glades occur on outcrops of the thin-bedded dolomite of several formations of the Canadian Series, particularly the Cotter and Powell. Their distribution follows the outcrop belt of these formations; glades and the similar bald knobs of south-central Missouri encircle the Ozark dome. On a smaller scale, their occurrence is determined by the presence of sufficient local relief in conjunction with the outcrop of thin-bedded dolomite. They are characterized by a thin soil cover, which is slightly acid and fairly high in organic matter, and by an extreme set of environmental conditions: saturation to the point of seepage in late fall and early spring, and desiccation during the summer months (Erickson, Brenner and Wraight, '42). The glade habitat appears to be an edaphic climax, rather than a stage in the succession to upland forest, or a product of a biotic influence, such as grazing by cattle. The red cedar, *Juniperus virginiana*, is the most characteristic tree associated with the glades; the glades can be recognized from a distance by the contrast which the dark green of the cedars offers to the surrounding broad-leaved forest. The red cedars occasionally form an open cover, but usually occur as scattered individuals and may even be absent. The dominant plant is clearly the bluestem, *Andropogon scoparius*, though there are other grasses, and several other species make a conspicuous seasonal show of flowers, such as *Leavenworthia uniflora*, *Houstonia angustifolia*, and particularly, *Rudbeckia missouriensis*. Many of the plants have xeromorphic characteristics. Flor-

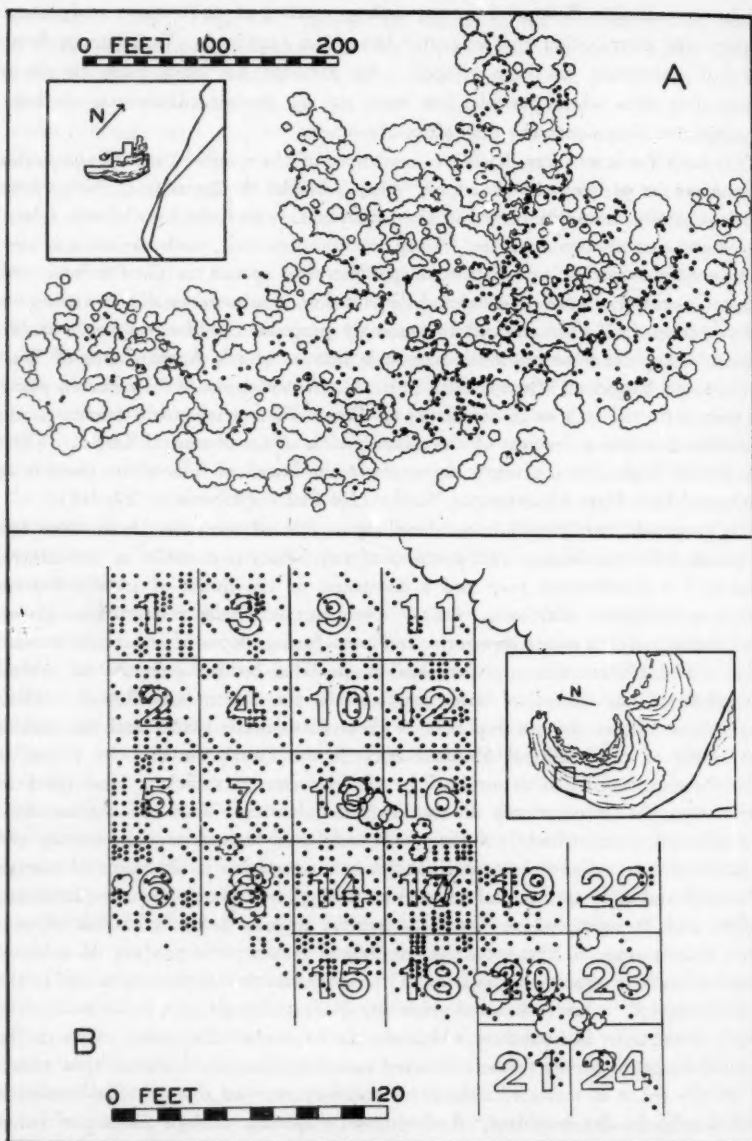


Fig. 1. Distribution of *C. Fremontii* var. *Rieblii* on greater part of small glade at R.2E, T.42N, S.10C (A); and on portion of larger glade at R.6E, T.39N, S.4-0 (B). Inserts show relation of area studied to glade as a whole. Small black dots represent *Clematis* plants, irregular outlines, trees. Domino effect in B is due to the fact that plants were counted in each 10-ft. quadrat, not plotted as they were for A. Numbered 40-ft. quadrats are referred to on p. 443 *et seq.*

istically, the glades are related to the shale barrens of the Appalachians (Wherry, '30), to the cedar glades of the Nashville Basin in Tennessee (Freeman, '33), to portions of the prairies of Kansas and Nebraska (Albertson, '37), and to glade-like grassy areas in the Arbuckle Mountains of Oklahoma and the Edwards Plateau of Texas.

DISTRIBUTION PATTERN

Because of its striking appearance, *Clematis Fremontii* var. *Rieblii* is a conspicuous member of the glade community, but in numbers it is subordinate. Its distribution on a number of glades has been studied in some detail. Figure 1 shows the distribution on the greater part of a small glade and a portion of a larger one that are not so much representative as illustrating approximately two extreme situations in which the plant is found. The maps were prepared from data obtained by laying out 10-ft. quadrats on the glades and plotting or counting the plants in each quadrat. On several other glades (fig. 2) the distribution has been studied by laying out 10-ft. transects of contiguous 10-ft. quadrats, usually at 250-ft. (50-pace) intervals, and normal to the "contour lines" formed by outcropping rock ledges.

Erickson and Stehn ('45) have published a statistical analysis of these data. They have pointed out that the data cannot be regarded as representing random (Poisson) distributions. Field observations suggested that the departure from randomness has its basis in a lack of uniformity of different portions of the glades as a habitat for *Clematis*. The data have been fitted by calculating two Poisson distributions for each glade, an "economic distribution," corresponding to suitable portions of the glade, and an "adventitious distribution," whose mean is small, representing unsuitable portions. The mean of the former is regarded as equivalent to Elton's ('32, '33) economic density.

The results of this statistical analysis should be considered in the light of field observations of conditions on the glades. The density counts are summarized in Table I. As contrasted with the tenfold variation in uncorrected, mean density, m_0 , the economic densities, m_1 , show a better agreement. The economic means of 1.02 and 1.10 plants per 100 sq. ft. are both for small glades; the rest, with means clustering around three or four plants per 100 sq. ft., apply to larger glades. The small glade at R.2E, T.42N, S.10C ($m_1 = 1.02$) is remarkable for its inaccessibility, and for the large size and number of red cedars. The data of line 6 in Table I ($m_1 = 1.10$) were obtained by combining data from two similar, adjacent glades in R.5E, T.40N, S.13. Both are small glades, though without such a conspicuous cover of red cedars as glade No. 1. The fact that the data appear to fall into two groups on the basis of economic density values is a reflection of the tendency, not recognized in the earlier field work, to select the larger, more "typical" glades for study. If more representative data were at hand, it would probably be found that the economic density is somewhat a function of the size of the glade, reaching an optimum value of three to four plants per 100 sq. ft. on large glades, and being smaller on smaller glades. It is thought that the conditions

TABLE I
DISTRIBUTION OF *CLEMATIS* ON GLADES
IN FRANKLIN CO. AND JEFFERSON CO., MO.
(Glade numbers correspond with those of fig. 2)

No.	Location	Area (acres)	Est. number of <i>Clematis</i>	Mean density (m_0)	Economic density (m_1)
1	R. 2E, T. 42N, S. 10C	2.8	1,140	0.57	1.02
2	R. 2E, T. 42N, S. 15B	13.4	6,780	1.16	3.61
3	R. 3E, T. 41N, S. 25	23.3	3,880	0.41	2.98
4	R. 4E, T. 40N, S. 15B	14.9	5,580	0.86	3.87
5	R. 4E, T. 40N, S. 15D	14.8	13,230	2.05	4.07
6	R. 5E, T. 40N, S. 13A	5.4	836	0.34	1.10
7	R. 5E, T. 40N, S. 13E	20.7	32,000	3.69	2.51
8	R. 6E, T. 39N, S. 4-0	24.3	11,300	2.85	3.76

of winter saturation and summer desiccation, etc., referred to by Erickson, Brenner and Wraight ('42), are developed to the extreme only on the largest glades, being somewhat ameliorated on smaller glades more closely surrounded by forest. *Clematis* may be limited to glades not because of its special adaptation to their physical environment, but because it finds competition from other species too severe elsewhere, as Salisbury ('29) has found to be the case for other plants of barrens, such as *Ranunculus parviflorus*. If that is so, *Clematis* would be expected to reach its optimum density on the large glades where biological competition is presumably least severe.

The implication of this statistical treatment, that the glades can be divided into two portions on the basis of their suitability for *Clematis*, deserves some amplification. A prominent physical characteristic of the glades is the occurrence at intervals of parallel outcrops of more massive rock than the thin-bedded dolomite which forms the glade proper. On the aerial photographs of the region, which were studied as a preliminary to the field work, these outcrops give the appearance of contour lines, and aid greatly in recognition of the glades. In the field the ledges are found to vary greatly in distinctness. *Clematis* characteristically occurs just below such a ledge of rock, though it is by no means strictly limited to such places. This and the fact that it seems to be more abundant near the lower edge of a glade suggest that one of the factors determining its presence is the amount of seepage water available during the spring. The unsuitable portions of the glade, or "blanks," are of at least three kinds: the exceedingly barren areas just above a ledge of massive rock, which are strewn with chert fragments and occupied almost exclusively by a sparse growth of the small grass, *Sporobolus*

beterolepis; very grassy portions, where *Clematis* would presumably meet severe competition with *Andropogon*; and small clusters of trees, *Juniperus virginiana*, *Bumelia lanuginosa*, *Cornus florida*, etc., which occur at intervals on the glades, often where a gully has developed.

It is apparent then that the distribution of *C. Fremontii* var. *RiehlII* on individual glades is characterized by considerable aggregation. The aggregates of plants are not well delimited, as can be seen by reference to fig. 1, but they do exist. They vary considerably in area, and they may include a few plants to a few hundred.

While the aggregates of plants are undoubtedly important in breaking up the population into local groups, the glades themselves, by their greater definiteness of outline and more complete isolation, must also be significant. On the distribution map (Erickson, '43b, fig. 2), about 15 negative records were plotted within the distribution area of the *Clematis*, with 160-odd positive records, indicating that roughly 87 per cent of the glades support some plants. Furthermore, the plant has never been found except on a glade, and it is probably justifiable, as a first approximation, to regard glades and colonies of plants as equivalent in examining the organization of the population. Those which have been carefully studied (Table I) vary in area from 2.8 to 24.3 acres, and in estimated number of plants from 830 to 32,000. However, it has been pointed out above that the sampling involved has not been satisfactory. Between 200 and 250 glades have been visited more briefly, and some impressions gained from that experience should be pertinent. The glades vary in area from about 80 acres (large glade two miles north of Platin) to small grassy areas which scarcely merit the name. In R.3E, T.41N, S.1-18, the total area in glades was measured by placing the tracings of the aerial photographs over a piece of paper ruled in small squares and counting the squares covered by glade outlines. Sixty glades were counted with a total area of 123 acres. Here, then, the average glade measures very nearly two acres in area. The number of plants per glade varies greatly, and probably corresponds only roughly with the area of the glade. Several glades of considerable area have been visited on which only one or a very few plants could be found. The upper limit in size of a colony is indicated by the figures in the fourth column of Table I, and the average size of a colony appears to be about 970, as calculated on page 422.

An impression of the degree of isolation between separate glades (= colonies) can be gained by examining fig. 2 and the larger scale map (fig. 3). On the whole, there is little difficulty in defining separate glades. It is apparent that the glades are not randomly distributed. No attempt has been made to treat this matter statistically, but obvious relations of the glades to the drainage pattern can be seen, as, for example, at R.4E, T.40N, S.11 and 14 (fig. 3) where the glades are ranged on either side of "branches" of Cotter Creek. Such topographically determined clusters of glades must also have significance in the subdivision of the population into local groups.

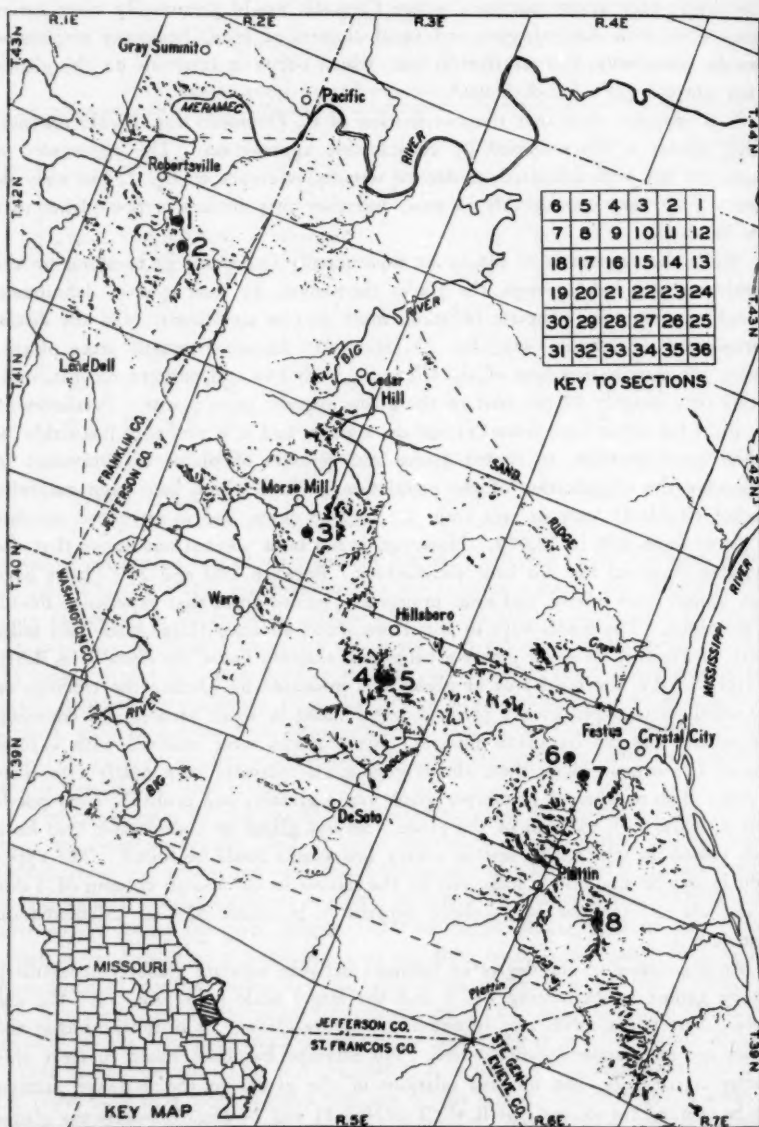


Fig. 2. Glades at which population density studies have been made. Glade numbers correspond with those of Table I.



Fig. 3. Two clusters of glades in R.4E, T.40N, S.10, 11, 14, 15, 22 and 23, illustrating their relationship to the drainage pattern. Figure is a reduction of tracings of aerial photographs. Width of figure is two miles.

The next higher category of organization is seen in fig. 2 as a tendency for the entire distribution range to fall into four *regions* of glade concentration: (A) south of Robertsville, (B) about Morse Mill, (C) south of Hillsboro and (D) about Plattin. Scattered glades occur outside these regions of concentration. It is probable that the factors responsible for this large-scale grouping of the glades are variations in thickness of the determining strata of thin-bedded dolomite and the amount of local topographic relief. The four regions appear to be about equivalent in total glade area, but one has the impression from field work that the plant is most abundant on the glades about Plattin, and least abundant in the vicinity of Morse Mill, with the Robertsville and Hillsboro regions intermediate.

TABLE II
HIERARCHY OF SUBDIVISIONS OF THE *CLEMATIS* POPULATION
(Compare with fig. 4)

Subdivision	Number	Total area (sq. mi.)	Glade area		Number of <i>Clematis</i>
			(sq. mi.)	(acres)	
Distribution range	1	436	7.0	4,460	1,500,000
Regions	4	100	1.5	980	300,000
Clusters of glades	50	—	0.09	60	30,000
Colonies (= glades)	1,450	—	—	2 (0.1-80)	970 (1-32,000)
Aggregates	15,000	—	—	0.2	97

It is thus seen that the distribution of *C. Fremontii* var. *Rieblii* falls naturally into a hierarchy of subdivisions, reminiscent of the hierarchy of subdivisions of the population of *Linanthus Parryae* which Wright ('43) devised for statistical reasons, but differing in that they have a natural basis and show no approach to equality in size. Some speculative calculations can be made of the relative size and number of the subdivisions (Table II). The estimate made in a previous paper (Erickson, '43b) of the total area over which *Clematis* is distributed stands; while several new records could now be added to the map, none are beyond the limits shown there. The calculations made in that paper of the total number of plants have been revised to include all the density data used in compiling Table I. The total was found to be 2,191,000, in gratifying agreement with the previously quoted estimate of 2,200,000 (rounded off from 2,197,000). However this figure has been reduced arbitrarily to 1,500,000, since the density counts weighted large glades too heavily. The estimate of the total number of glades, 1450, has been calculated by assuming that the 60 glades counted in the 18 sq. mi. at R.3E, T.41N, S.1-18, can be considered representative of the entire area. The average number of plants per glade, 970, has been obtained by dividing the number of glades into the total number of plants for the entire area. Similar calcula-

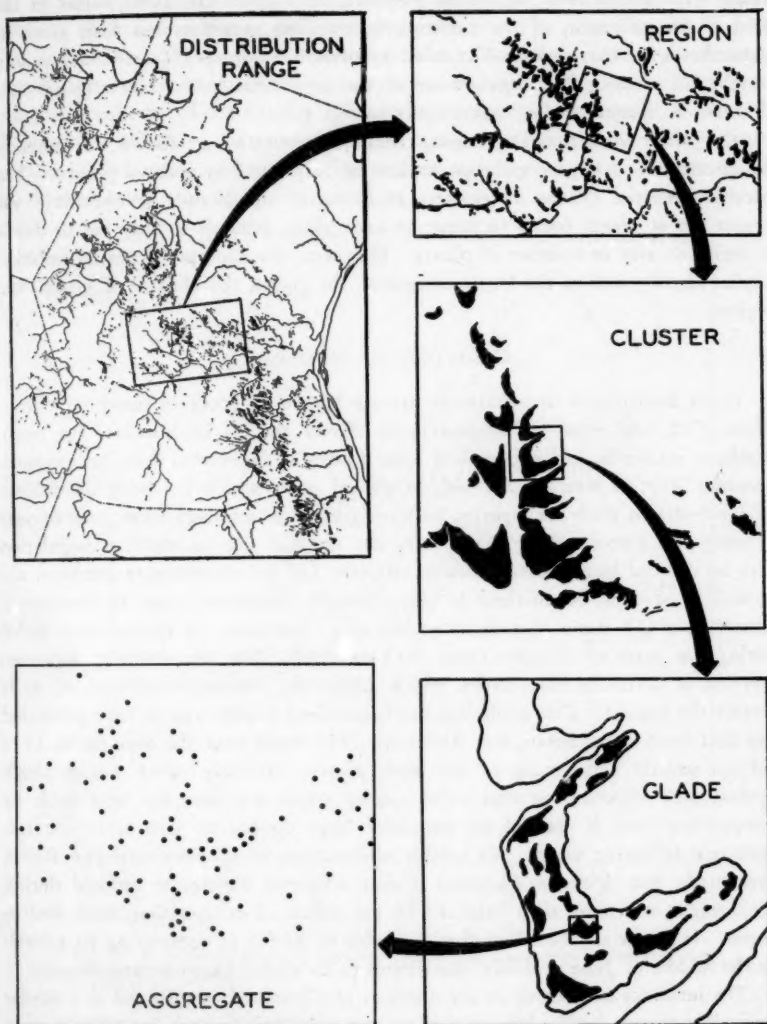


Fig. 4. Diagram to illustrate organization of the distribution range of *C. Fremontii* var. *RiehlII* into a hierarchy of subdivisions: regions of glade concentration; clusters of glades; glades; and aggregates of *Clematis* on glades. Compare with Table II.

tions, with liberal rounding-off of numbers, have given the other values in the table. An indication of the variation in numbers of plants has been given in parentheses for the glades. The other subdivisions also vary greatly in area and number of plants. The organization of the population into a hierarchy of subdivisions is illustrated diagrammatically in fig. 4.

On purely geographical grounds, then, *C. Fremontii* var. *Rieblii* can properly be described as a large population broken up into partially isolated groups. The partially isolated groups of greatest evolutionary significance are probably the aggregates of plants found to occur on each glade, difficult as they are to define in terms of area or number of plants. However, the concept of partial isolation applies equally well to the larger categories, the glades, the clusters of glades, and regions.

CONSTANCY OF NUMBERS

Great fluctuations in population size are known to occur in many organisms. Elton ('42, and other publications) has shown this to be the case for many northern mammals, and it is true of some species of *Drosophila* (see, for example, Spencer, '41). *Linanthus Parryae*, an annual plant which has been the subject of a population study, is reported to vary greatly in numbers from year to year (Epling and Dobzhansky, '42). Since the smallest size to which a population may be reduced largely determines its effective size for evolutionary purposes, the possibility of such fluctuations in this *Clematis* population must be considered. Albertson ('42) states that many plants of *C. Fremontii* in Kansas were killed during the years of drought from 1933 to 1939. The late drought, however, was not so severe in the Ozarks, which adjoin the Mississippi embayment, as it was on the prairies. This study was not begun long enough ago to have permitted any first-hand observations, but Anderson ('43) states that the drought of 1936 did not greatly harm many of the glade plants. Its main effect was to check *Andropogon scoparius*, so that other species which are normally held back by competition with it showed an unusually large display of flowers in the immediately following years. No specific observations of *C. Fremontii* var. *Rieblii* were made, but Anderson's opinion is that whatever damage it suffered during the drought was more than balanced by the release of competition from *Andropogon*. It might also be added that the habit of *Rieblii* of completing its growth by the middle of June probably contributes to its ability to withstand drought.

The influence of grazing on the numbers of *Clematis* is manifested in a similar way. The leaves, besides being very leathery when mature, are exceedingly acrid (Greshoff, '09, reports the presence of hydrocyanic acid in *C. Fremontii*), and cattle avoid them. The only evidence of disturbance by livestock is an occasional young shoot which has been nipped off when an inch or so above ground, presumably by error, and flowers which are occasionally removed without disturbance to the leaves. Grazing, however, keeps back the grasses, such as *Andropogon*, and the ultimate effect is to allow *Clematis* to increase both in numbers and in the

size of individual plants. This is strikingly seen in some cases where a fence divides a glade into a grazed and ungrazed portion. The plants on the grazed portion are noticeably larger, and flower somewhat earlier than those on the ungrazed part. Another biotic factor may be mentioned. The plant is subject to sporadic attacks by blister beetles, *Epicauta marginata*, which devour the leaves. Their attacks, however, are merely an annoyance to the investigator. They occur too late in the season, and are not frequent enough, to influence the population size of the plant seriously.

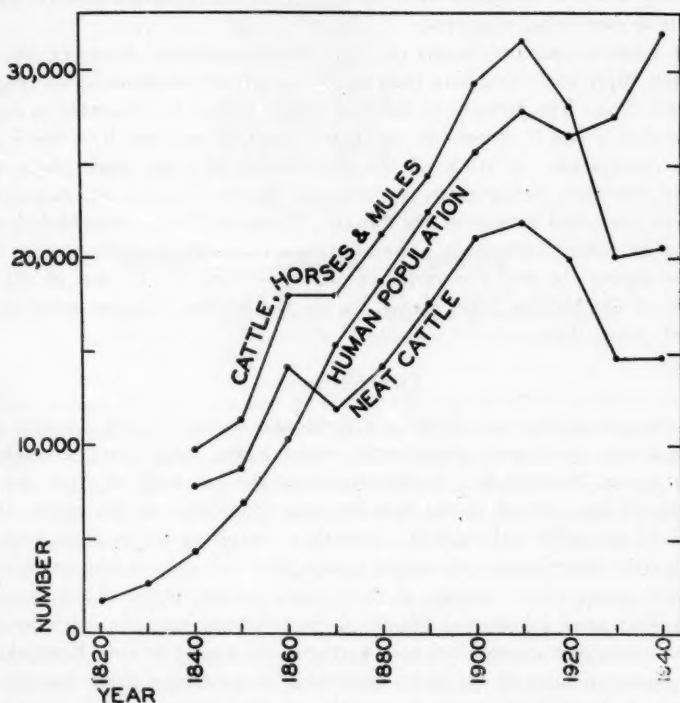


Fig. 5. Data on numbers of livestock and human population in Jefferson Co., Mo., from U. S. Census.

It is presumed that *Clematis* has increased in numbers since the white settlement of the country. United States Census data (fig. 5) show that the livestock population of Jefferson County reached a maximum in 1910, with a considerable decline until a minimum was reached in 1930, since when there has been an increase. Because the numbers of plants is believed to have varied roughly in proportion to the severity of grazing of the glades, it may be concluded that the population size of *Clematis* has increased considerably since 1800, and that it now

has reached relative stability, subject to fluctuations in relation to general economic conditions and changes in the management of individual farms. It is thought that the perennial habit of the plant may serve to damp such influences. Unfortunately, no direct evidence is at hand. A study of the old records of collection of the plant (Erickson, '43b) makes it seem probable that no conspicuous extension or restriction of range has occurred since the 1880's. Certainly no large-scale fluctuation in numbers has occurred in the four years during which the author has observed the plant. On the whole, the size of individual colonies appears remarkably stable as compared with the spectacular fluctuations which are known to occur in some other organisms.

The apparent constancy in size of this *Clematis* population at present does not, of course, imply that there have been no restrictions or extensions of its range in geological time. The presence of the very closely related *C. Fremontii* in Kansas suggests that it and *C. Fremontii* var. *Rieblii* must at one time have had a continuous distribution. A study of the distributions of other glade plants, particularly *Oenothera missouriensis*, suggests that the two *Clematis* populations may have been connected by way of the Edwards Plateau of Texas (unpublished maps prepared by Edgar Anderson). The separation into two populations may have occurred during the semi-arid period of late Pleistocene, or, in view of the importance of competition from grasses, during the warmer, moister period which followed (Sears, '35).

LIFE HISTORY

Clematis Fremontii var. *Rieblii* is a herbaceous perennial with a woody stem and remarkably coriaceous, prominently veined leaves, which have inspired the common name, "leatherleaf." It flowers during the last week of April and the first week of May, though it was seen flowering sporadically in September, 1941, a month of unusually high rainfall. Growth is completed within three weeks or a month after flowering, and the plants remain green for eight or ten weeks, turning brown during July. Because of their woody nature, many of the stems remain in place until February or March of the following year, the leaves by that time having become skeletonized and weathered to attractive gray laceworks of veins. However, some of the plants have been blown free of their moorings by October. A large plant forms a roughly spherical mass of rigid stems and leaves, and when it is freed, it may be carried for some distance over a glade as a tumbleweed.

The persisting structure is a woody caudex (fig. 6), provided with a mass of brown fleshy roots, in which the reserve food is starch. Two or four lateral buds are formed in the fall at the lower nodes of the old stem, one or more of which unfolds the next spring to form a new shoot. This process, over a period of years, gives rise to a certain amount of branching of the caudex, the older portions of which are torn apart by the growth of the roots. A large plant may consist of perhaps 20 shoots, arising from four or five separate caudices. Branching of the



Fig. 6. Caudex of *C. fremontii* var. *rieblii*, which supported two shoots in 1943. It was collected in June, and lateral buds had not yet developed. Fleshy roots have been removed. Scale in centimeters.

caudex apparently does not provide a very efficient means of vegetative propagation. Morphological variation from plant to plant is sufficient to permit genetic individuals to be distinguished with some certainty. In a careful examination of perhaps 200 plants for evidences of clonal reproduction, only one case was found in which two separate plants appeared to belong to the same clone. They were about one foot apart, and there was evidence that the separation was accidental, caused by the fall of a tree trunk over the original clump. Three or four clumps were found which were actually two plants. In the population density studies reported above, each plant (or clump) old enough to have flowered has been scored as an individual regardless of its size or number of shoots.

In most species of *Clematis* the achenes are provided with conspicuous plumose tails, presumably well adapted to wind dispersal (fig. 7). In *C. fremontii* var. *rieblii*, however, the achene-tails are naked for the greater part of their length, though their basal portions and the apices of the achenes are silky (fig. 8). They are not suited for wind dispersal in the usual sense. Dispersal by the fur of a mammal is hard to visualize, and no evidence has been seen of their use as food by a bird or mammal. Some dissemination is probably achieved by the tumbleweed habit of the largest plants, but most of the achenes merely fall to the base of the parent plant. Dispersal of the achenes over a single glade is probably adequate, but the transportation of achenes from one glade to another must be a rare occurrence.

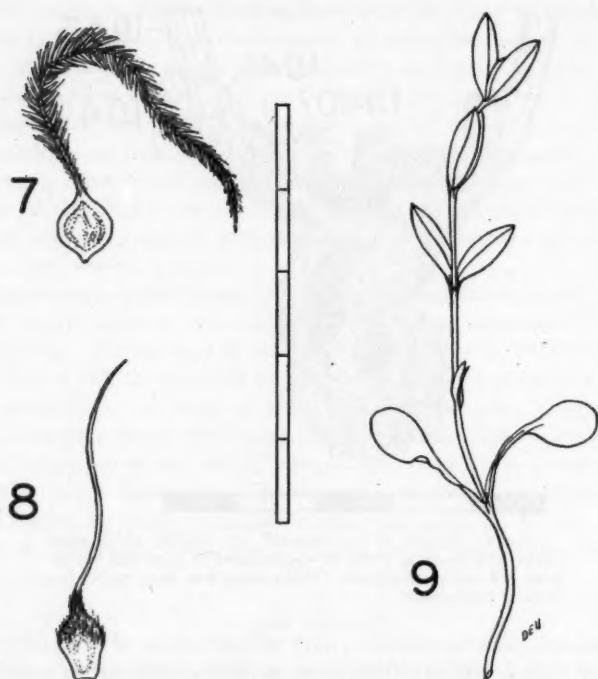


Fig. 7. Achene of *C. Viorna*, showing plumose achene-tail. Fig. 8. Achene of *C. Fremontii* var. *Rieblii*, showing naked achene-tail. Fig. 9. Seedling of *C. Fremontii* var. *Rieblii*, perhaps one month after germination. Scale in centimeters.

Attempts to germinate seeds under greenhouse conditions have been largely unsuccessful. Of about 600 seeds planted, three have germinated. Better success would perhaps be had by layering, but the experiments have not been carried out. Indications are that the percentage of germination of seed in the field is also low. The seedlings escaped detection in the early field work, because of their minute size (fig. 9). Many small sterile plants were found, but examination of the caudex always showed them to be two or more years old. When the seedlings were finally recognized, little trouble was had in finding them on any glade where careful search was made. They are not abundant. Presumably achenes are dropped in the vicinity of every large plant every fall; groups of seedlings can be found in such places in perhaps one case in 100. The conditions required for germination are not well understood, but adequate shade appears to be one of them. Germination takes place in the spring. Seedlings have not been found in September and October, though these months are often characterized by warm rainy weather, similar to that of April.

Growth of the plants seems to be quite slow, four or five years apparently being required from germination until the first flower is produced. Young, sterile plants (fig. 10) have been found on all the glades which have been studied. At R.4E, T.40N, S.15D, 61 of the 528 plants counted, or 8.7 per cent, were such plants. The conclusion that four or five years are required before flowering is based on examination of the caudices of many sterile plants, and of young plants which have produced a single flower. It is more difficult to estimate the age of larger clumps, since the older portions of a caudex are badly fragmented, and annual rings in the wood of the caudex are quite indefinite. Deduction from the size of the plant and of the caudex places the age of large clumps, such as illustrated in fig. 11, at 15 or 20 years, though there is no reason for thinking that they may not be indefinitely older.

POLLINATION

Since vegetative reproduction and seed dispersal are quite inefficient, pollen transportation must be looked to as the principal means of gene exchange from one glade to another, and perhaps also from one portion of a glade to another. The flowers are insect-pollinated. They are protogynous, as will be seen from fig. 12, and produce nectar at the base of the stamens. In view of these facts, cross-pollination would seem to be the rule, and more will be said about that below. However, the filaments elongate after the anthers have dehisced, and in an old flower the inner anthers are in contact with the style-tips, so that self-pollination is at least mechanically possible. Glassine bags have been placed over a number of flowers before anthesis to determine the seed-set in enforced self-pollination. The results have been nearly inconclusive. In the first attempts the bags were fastened around the peduncles of flowers, and with one exception failed to stay in place. When the bags were placed over several leaves as well as the flower, the plant and bag were blown over in the wind, became wet, and in most cases molded. Of 106 bags which were placed and later collected, three contained a full head of achenes, and three a few seeds each. The failures to set seed are attributed to the injury done the plant by enclosing it. Normally seeds are set by all the flowers except the smallest ones which occur late in the season on weak branches. Tentatively it is perhaps safe to assume that a plant will be self-pollinated if cross-pollination does not occur first.

Clematis is visited by a variety of insects while it is in flower. An insect net was carried for seven days during April, 1943, and as many as possible of the insects found on the flowers were captured. The specimens have been identified by Mr. Harold I. O'Byrne, and Mr. Richard Froeschner, with the exception of some smaller Hymenoptera. The data are presented in Table III. The most frequently found insects are four species of Pentatomidae. They are typically found lurking at the base of a flower, often with the proboscis inserted into one of the fleshy sepals. It is doubtful whether they are concerned in pollination, since they rarely venture to the opening of the flower, and apparently do not move from one plant to another often. The most conspicuous visitors, in order of the

TABLE III
INSECTS COLLECTED ON *CLEMATIS* FLOWERS

Order Family Species	Number of specimens		
	♂	♀	Total
Homoptera			
Cicadellidae			
<i>Oncometopia lateralis</i> (Fab.)		1	1
Hemiptera			
Pentatomidae			
<i>Euschistus variolarius</i> (Beauv.)	19	3	22
<i>Eu. euschistoides</i> (Voll.)	4	1	5
<i>Thyanta custator</i> (Fab.)	2	2	4
<i>Peribalus limbolaris</i> Stål.		1	1
Neididae			
<i>Neides muticus</i> (Say)	2	2	4
Lepidoptera			
Papilionidae			
<i>Papilio ajax</i> Linn.	1	1	2
<i>P. troilus</i> Linn.		2	2
<i>P. philenor</i> Linn.	1	1	2
Lycanidae			
<i>Strymon melinus</i> Hbn.	1		1
<i>Everes comyntus</i> (Godt.)	1	1	2
Hesperiidae			
<i>Proteides clarus</i> (Cram.)	2	1	3
<i>Thorybes pylades</i> (Scud.)		1	1
<i>Tb. batyillus</i> (Ab. & Sm.)	3		3
<i>Erynnis brizo</i> (Bdv. & Lec.)	1		1
Sphingidae			
<i>Hemaris diffinis</i> (Bdv.) form <i>tenuis</i> Grote	5	6	11
Coleoptera			
Dermestidae			
<i>Cryptorhopalum picicorne</i> Lec.			1
Melyridae			
<i>Collops vicarius</i> Fall	1		1
Hymenoptera			
Bombidae			
<i>Bombus impatiens</i> Cresson		2	2
<i>B. americanorum</i> (Fab.)		3	3
Apidae			
<i>Apis mellifica</i> Linn.			1
Unidentified			
Hymenoptera (5 species ?)			12
Arachnida, Thomisidae			15

frequency with which they have been seen on the flowers, are the hawk moth, *Hemaris diffinis*, the bumblebees, *Bombus impatiens* and *B. americanorum*, and the swallowtails, *Papilio ajax*, *P. troilus* and *P. philenor*. They alone of the insects captured have proboscides long enough to reach the nectaries from the opening of the flower, a distance of about two cm. It is doubtful whether *Hemaris* or the *Papilio* species are involved in pollination to a considerable extent. The manner in which they cling to the recurved tips of the sepals while obtaining nectar suggests that they may be able to visit many flowers without picking up



Fig. 10. Plant of *C. Fremontii* var. *Rieblii* estimated to be four years old. Scale in centimeters. Fig. 11. Mature plant of *C. Fremontii* var. *Rieblii* probably 15 years old or older. Scale in centimeters. Fig. 12. Flower of *C. Fremontii* var. *Rieblii*. Note that styles are exerted. Anthers have not yet dehisced.

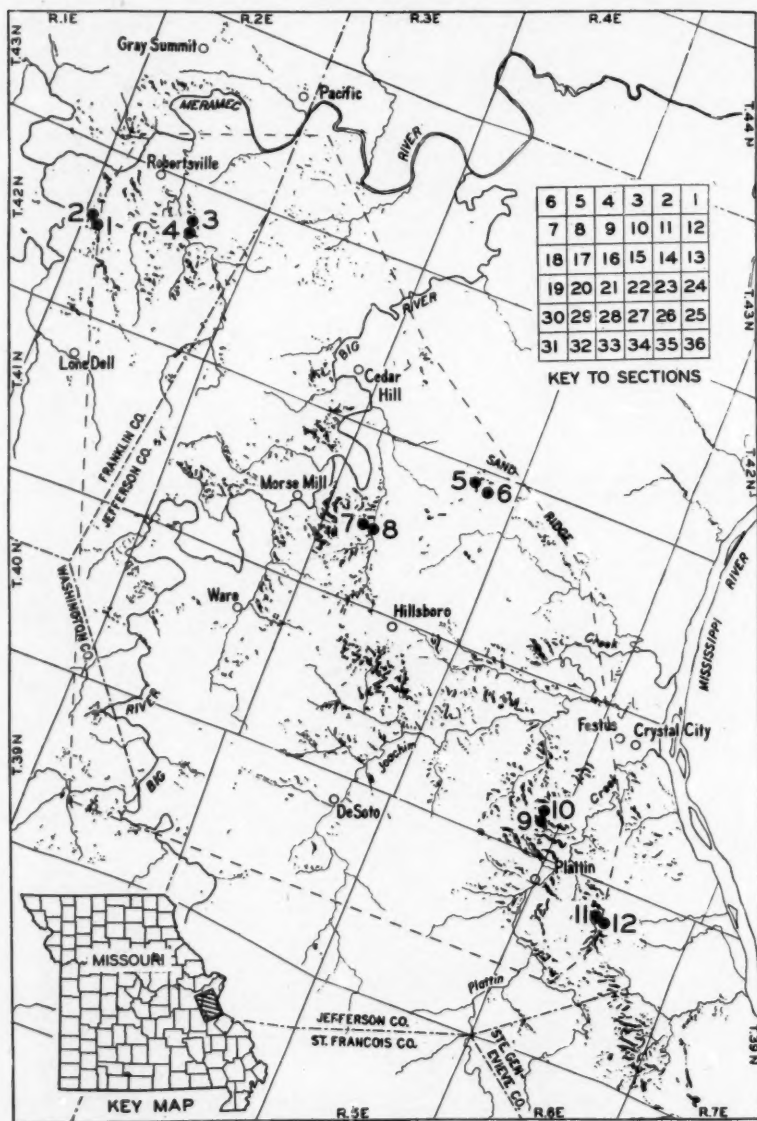


Fig. 13. Glades at which frequency of colored sepal tips has been determined. Glade numbers correspond with those of Table IV.

much pollen. No pollen grains have been detected on the pinned specimens with a hand lens. The bumblebees are undoubtedly queens who have recently come out of hibernation. In late April, they have just begun the establishment of nests (Frison, '27), and are engaged in collecting nectar rather than pollen. This is borne out by the fact that the corbiculae of all the specimens are empty. However, some pollen has been found clinging to the hairs of the head and the prothoracic legs of all of the pinned specimens. It is easy to understand how the bumblebees pick up this pollen. Their behavior at the flowers is much cruder than that of the hawk moths and the swallowtails. Instead of hanging daintily from the sepal tips and probing discretely for nectar, a bumblebee appears to be struggling in an attempt to ram its entire head into the flower as far as possible. The visits of the smaller butterflies of the *Lycaenidae* and *Hesperiidae* were puzzling at first. It was obvious that they are unable to reach the nectar by the normal route. Closer observation of several individuals showed that they insert their proboscides at the base of the flower, between the valvate margins of two sepals. By this means, of course, they completely avoid contact with the pollen. Old flowers, from which the sepals are about to drop, often swarm with black ants. The ants undoubtedly come in contact with pollen, but it is doubtful whether a single ant visits many flowers in a short period of time, or visits flowers which are young enough to have receptive styles. The honeybee specimen, *Apis mellifica*, and several of the unidentified smaller bees are well loaded down with pollen. They and the bumblebees are certainly the most important pollinators of this *Clematis*. Other species listed in Table III are probably accidental visitors; they could have been collected more efficiently by sweeping. An interesting sidelight on the insect relations of *Clematis* concerns the crab spiders (*Thomisidae*), of which 15 specimens were obtained. The writer was fascinated on one occasion to watch a *Hemaris* hovering before a flower, and to see it attacked and killed by a spider which had been waiting at the base of the flower.

During the 1942 season some notes were taken on the frequency of insect visits to the flowers. The observations were made without the disturbance caused by attempts to capture the visitors, and were incidental to other work. In an estimated 15 hours on seven different glades, during which an average of perhaps 20 plants were under close enough observation to insure detection of a pollinating insect, nine bumblebees were observed to visit a total of 24 flowers, two honeybees visited one flower each and flew out of sight, one small bee was observed on a single flower, five *Papilio*s visited a total of 17 flowers, four *Hemaris* were observed, and one visit by an unidentified smaller butterfly was made. In all of the observations of insects, the writer has been impressed with the great variation from one glade to another. For instance, few hawk moths were recorded in 1942, while in 1943, when other glades were visited, they appeared to be the most frequent visitors, mainly because of the large numbers encountered on a single glade at R.2E, T.42N, S.15B. If any reliance can be placed on the crude estimates made above, it would seem that there is ample provision for the cross-

pollination of a plant within two days of anthesis. Actually, the frequency of pollination is probably higher; the smaller bees were not recorded because their visits failed to attract the writer's attention from other activities and no observations of nocturnal insects were made. Since the bumblebees and the honeybees are reputed to forage over wide areas, the occasional transport of pollen from one glade to another seems quite probable. While working on a single glade, the bumblebees do not systematically go from one flower to its nearest neighbor, but may fly several yards between visits. In a large colony of *Clematis* it seems probable that the circle of possible mates for a given plant may well include a few hundred individuals.

PATTERN OF DIFFERENTIATION

Data on the distribution of gene frequencies within a population provide the most useful information for evaluating the relative roles of selection and random differentiation. However, the collection of such data presupposes a basic fund of knowledge of the genetics of an organism which exists in relatively few cases. Lacking that for *Clematis*, a careful examination of many plants has been made for a morphological character which can at least be scored as present or absent, in the hope that eventually it might turn out to have a simple genetic basis. There is considerable variation in flower color, the outer surfaces of the sepals ranging from the blue and purple of the manuals, to practically white. Most flowers in anthesis are nearly white, with considerable variation in the distribution of the small amount of color which is present. It is suspected that true albino flowers exist, but they cannot be distinguished with certainty from those in which the pigment is very dilute.

The inner (adaxial) surfaces of the recurved sepal tips, however, show a discrete variation in color which is suggestive of a simple mode of inheritance, and a number of plants have been scored for presence or absence of color (pink or blue) at this place. A collection of 36 or fewer flowers was made on each of 12 glades, so selected that they could be arranged in pairs. The two glades of a pair are on adjacent ridges (fig. 13) about 0.35 mi., or 1850 ft., apart on the average. Two pairs of collections, 4.1 mi. apart on the average, were made in each of three regions. Glades 1-4 in the Robertsville region are about 28.5 mi. from glades 9-12 in the Platin region, and glades 5-8, in the Morse Mill region, are midway between. The number and proportion of flowers with colored sepal tips in each collection, in each pair of collections, and in each region are shown in Table IV. The proportions for the three regions, 0.36, 0.11, and 0.05, suggest a "cline" (Huxley, '38), the frequency of colored sepal tips being greatest in the Robertsville region and decreasing toward the southeast.

In examining the data statistically, the assumption that the population is really uniform in proportion of colored sepal tips has first been tested by the χ^2 test. Theoretical frequencies of colored sepal tips have been calculated by multiplying the total number of flowers in each collection by the over-all proportion,

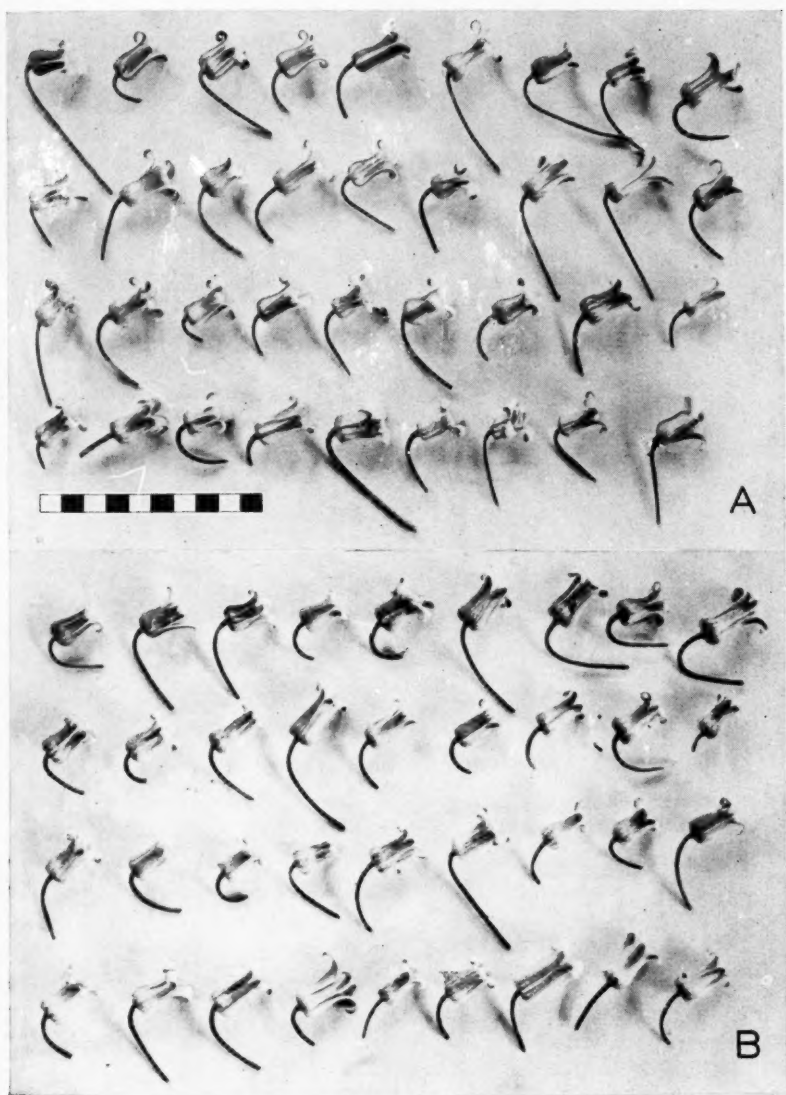


Fig. 14. Two collections of flowers of *C. fremontii* var. *riehlII*, obtained at R.2E, T.42N, S.18H (A), and at R.6E, T.39N, S.4P (B), to illustrate nature of morphological variation. Scale in centimeters.

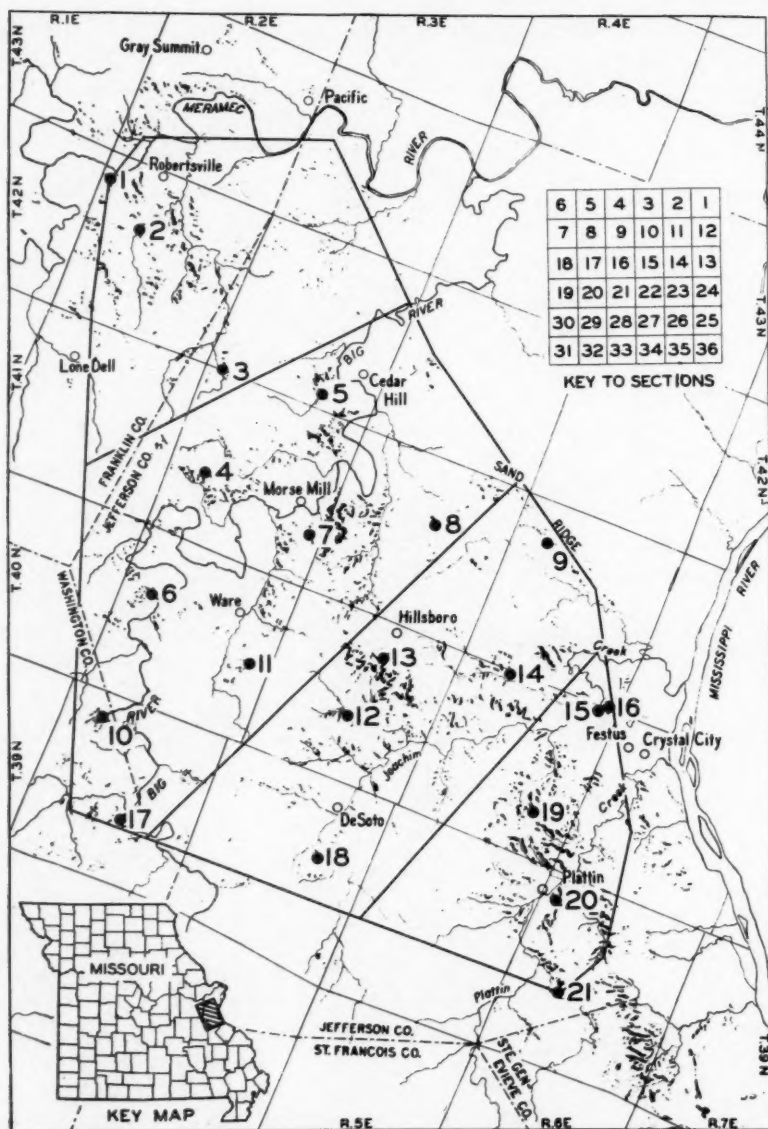


Fig. 15. Glades at which measurements have been made of sepal length, sepal width, margin width, and sepal coil. Glade numbers correspond with those of Table V.

TABLE IV
FREQUENCY OF COLORED SEPAL TIPS
(Glade numbers correspond with those of fig. 13)

No.	Location	Number of flowers			Number and proportion with colored tips			Significance of difference between proportions				
		Glade	Pair	Region	Glade	Pair	Region	Betw. glades		Betw. pairs		
								x	2P	x	2P	
1	R. 2E, T. 42N, S. 18D	31	67	139	11-0.36	26-0.39	50-0.36	0.52	0.61	0.67	0.50	
2	R. 2E, T. 42N, S. 18H	36			15-0.42							
3	R. 2E, T. 42N, S. 10E	36			11-0.31							24-0.33
4	R. 2E, T. 42N, S. 10K	36			13-0.36							
5	R. 4E, T. 41N, S. 2E, F	18	54	93	3-0.17	7-0.13	10-0.11	0.57	0.58	0.81	0.42	
6	R. 4E, T. 41N, S. 2G	36			4-0.11							
7	R. 4E, T. 41N, S. 20D	14			2-0.14							3-0.08
8	R. 4E, T. 41N, S. 20E	25			1-0.04							
9	R. 5E, T. 40N, S. 25F	36	72	144	2-0.06	3-0.04	7-0.05	0.59	0.56	0.39	0.70	
10	R. 5E, T. 40N, S. 25B	36			1-0.03							
11	R. 6E, T. 39N, S. 4P	36			3-0.08							4-0.06
12	R. 6E, T. 39N, S. 4S	36			72							

Significance of difference between proportions, between regions:

Betw. glades 1-4 and 5-8: $x = 4.30$; $2P = 1.6 \times 10^{-5}$

Betw. glades 1-4 and 9-12: $x = 6.52$; $2P = 1.1 \times 10^{-10}$

Betw. glades 5-8 and 9-12: $x = 1.72$; $2P = 8.6 \times 10^{-2}$

Betw. glades 1-4 and 5-12: $x = 7.05$; $2P = 2.0 \times 10^{-12}$

0.178. Carrying through the calculation gives a χ^2 value of 43.9, with 8 degrees of freedom. The probability for a higher χ^2 value is less than 0.001, which rules out the possibility that the population is uniform in this character. (In this statistical analysis, and the succeeding ones, the methods and orthography of Rider ('39) have been followed except in the analysis of covariance on p. 446 *et seq.*)

For a more detailed analysis of the data, calculations have been made of the significance of the difference between the proportions for each pair of glades, for the two pairs of glades in each region, and for the three regions. There is no significant difference in proportion of colored sepal tips between any two adjacent glades (Table IV, third column from right), nor between the two pairs of glades in each region (Table IV, last column). The difference in proportion between the Robertsville and Morse Mill regions, between Robertsville and Plattin, and between

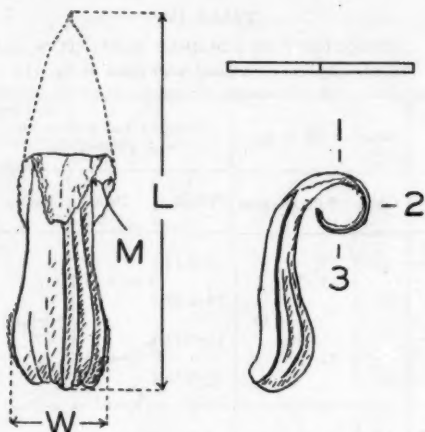


Fig. 16. Sepal of *C. Fremontii* var. *Rieblii*. Drawing at left illustrates manner in which measurements of sepal length, *L*, sepal width, *W*, and margin width, *M*, were made. Drawing at right illustrates method of scoring "sepal coil" in quadrants. Scale in centimeters.

the Robertsville region and the remaining glades, however, is highly significant (Table IV, bottom). Whether there is a real difference between the Morse Mill and Platin regions is doubtful. The northwest-southeast differentiation in this character may be described as a cline, but its most significant feature is the deviation of the plants of the Robertsville region.

The flowers were brought to one place so that they could be studied at one time and photographed. Examination of the flowers indicated that the differentiation in proportion of colored sepal tips is correlated with similar differentiation in the color of the entire sepal. Perhaps it is merely an expression of the latter.

The flowers also show evident differences in a number of continuously varying characters, as illustrated in fig. 14. Measurements of four such characters have been made on another series of glades. The 21 glades at which measurements, usually of 35 flowers, were made are indicated in fig. 15. They are scattered throughout the distribution range. Measurements of the distance between each of the 210 pairs of glades have been made with dividers on a map (scale: $\frac{1}{4}$ in. = 1 mi.), averaged, and the average converted to miles. This yields an average distance between the glades of 12.8 mi.

In making the flower measurements, care was taken to select flowers only from the primary shoots of mature clumps. The flowers which terminate the primary shoots of a single clone are remarkably similar in size, coloration, and general aspect. Those which terminate secondary branches are often smaller, later in anthesis, and darker in color. None of the latter have been included in the measurements. The sampling scheme has been to select flowers from a re-

TABLE V
MEASUREMENTS OF FLOWERS
Means and Standard Deviations for Glades
(Glade numbers correspond with those of fig. 15)

No.	Location	N	Sepal length, mm.		Sepal width, mm.		Margin width, mm.		Sepal coil quadrants	
			\bar{X}	σ^*	\bar{X}	σ^*	\bar{X}	σ^*	\bar{X}	σ^*
1	R. 2E, T. 42N, S. 7A	45	33.56	2.93	8.84	0.54	2.03	0.47	3.29	1.05
2	R. 2E, T. 42N, S. 17B	37	34.08	3.86	9.51	1.04	2.46	0.57	3.41	1.12
3	R. 3E, T. 42N, S. 31D	35	37.54	4.92	10.00	0.98	1.87	0.70	3.06	0.88
4	R. 3E, T. 41N, S. 20A	35	34.20	3.95	9.31	0.93	1.74	0.51	3.46	0.98
5	R. 3E, T. 42N, S. 35B	35	33.09	4.10	9.31	1.07	1.70	0.43	3.31	0.80
6	R. 3E, T. 40N, S. 17A	35	34.20	3.47	10.11	0.97	1.73	0.41	2.74	0.79
7	R. 3E, T. 41N, S. 25D	35	34.66	2.92	9.83	1.19	1.89	0.42	3.69	0.55
8	R. 4E, T. 41N, S. 15B	35	33.14	3.38	10.11	1.15	2.00	0.42	3.20	0.60
9	R. 5E, T. 41N, S. 8B	32	29.72	5.92	9.06	1.08	1.69	0.47	3.34	0.84
10	R. 3E, T. 39N, S. 5D	35	32.91	4.02	9.54	1.39	1.66	0.60	2.97	0.79
11	R. 3E, T. 40N, S. 24B	35	34.46	3.72	9.34	1.03	2.06	0.64	2.63	0.87
12	R. 4E, T. 40N, S. 22C	35	35.06	3.10	10.14	1.28	1.76	0.43	3.11	0.84
13	R. 4E, T. 40N, S. 10E	35	34.37	4.06	9.80	0.97	1.70	0.41	2.80	0.69
14	R. 5E, T. 40N, S. 5D	35	33.80	3.34	9.54	1.19	1.67	0.49	2.77	0.87
15	R. 5E, T. 40N, S. 1B	35	32.37	2.74	9.37	1.28	1.89	0.41	2.89	0.95
16	R. 5E, T. 40N, S. 1C	35	32.66	3.73	9.49	1.09	1.90	0.53	2.71	0.90
17	R. 3E, T. 39N, S. 22B	35	34.60	3.59	10.26	1.21	2.14	0.55	3.00	1.00
18	R. 4E, T. 39N, S. 14A	35	36.57	3.40	10.23	1.48	2.09	0.55	3.17	0.96
19	R. 5E, T. 40N, S. 26B	35	33.89	7.02	9.69	1.96	1.74	0.53	2.71	1.17
20	R. 6E, T. 39N, S. 6J	35	35.97	4.00	9.49	4.09	2.01	0.44	3.14	0.99
21	R. 6E, T. 39N, S. 20B	35	33.34	2.89	9.31	1.12	1.64	0.43	2.97	0.83
Total		744	34.02	4.18	9.63	1.28	1.88	0.53	3.07	0.94

stricted portion of each glade, rather than to sample the entire population of the glade. Usually the measurements were begun at a point where the plants were abundant, and a roughly spiral course was followed, during which a flower from each mature plant encountered was measured. No records were kept of the location on the glade of the plants selected. A sepal was removed from each flower, and the measurements indicated in fig. 16 were made with a celluloid rule. The length of the sepal was measured to the nearest mm., after straightening the recurved tip, but no attempt was made to flatten the thick base. Width was

TABLE VI
MEASUREMENTS OF FLOWERS
A. Analysis of Variance for Glades

CHARACTER	Sum of squares of deviations	Degrees of freedom	Mean square deviation	<i>w</i>	<i>P</i>
SEPAL LENGTH					
Within glades	11,271.14	723	15.59	5.51	< 0.0001
Among glades	1,717.64	20	85.88		
Total	12,988.78	743			
SEPAL WIDTH					
Within glades	1,158.92	723	1.603	3.60	< 0.0001
Among glades	115.46	20	5.773		
Total	1,274.38	743			
MARGIN WIDTH					
Within glades	202.65	723	0.2803	5.52	< 0.0001
Among glades	30.96	20	1.5480		
Total	233.61	743			
SEPAL COIL					
Within glades	658.84	723	0.9113	3.16	< 0.0001
Among glades	57.66	20	2.8833		
Total	716.50	743			

B. Analysis of Variance for Regions

SEPAL LENGTH					
Within regions	12,906.32	740	17.44	1.58	0.20
Among regions	82.46	3	27.49		
Total	12,988.78	743			
SEPAL WIDTH					
Within regions	1,257.78	740	1.700	3.26	0.020
Among regions	16.60	3	5.533		
Total	1,274.38	743			
MARGIN WIDTH					
Within regions	224.85	740	0.3039	9.61	< 0.0001
Among regions	8.76	3	2.9200		
Total	233.61	743			
SEPAL COIL					
Within regions	705.44	740	0.9533	3.87	0.0089
Among regions	11.07	3	3.6887		
Total	716.50	743			

measured, to the nearest mm., at the widest point, quite near the base of the sepal, without any attempt to flatten it. The width of the expanded sepal margin was measured to the nearest 0.5 mm., at its widest point, usually quite near the tip of the sepal. The degree to which the tip of the sepal is recurved, "sepal coil," was scored by noting the number of quadrants through which the tip has moved in anthesis. Thus, if the sepal tip has turned through 360°, as has the one illustrated, it is scored as 4. The mean and standard deviation of each series of measurements are given in Table V. Because of the relative coarseness of the scale used for three of the measurements (width, margin and coil), Sheppard's correction has been applied in calculating the standard deviations.

Inspection of the table discloses differences in means from one glade to another in each of the characters. In order to determine whether the variation in these characters from one glade to another is greater than that on a single glade (in

TABLE VII
MEASUREMENTS OF FLOWERS

A. Means and Standard Deviations for Regions

Region	N	Sepal length, mm.		Sepal width, mm.		Margin width, mm.		Sepal coil, quadrants	
		\bar{X}	σ^*	\bar{X}	σ^*	\bar{X}	σ^*	\bar{X}	σ^*
Robertsville. Glades 1, 2, 3	117	34.90	4.41	9.40	1.24	2.12	0.62	3.26	1.03
Morse Mill. Glades 4, 5, 6, 7, 8, 10, 11, 17	280	33.91	3.72	9.73	1.19	1.86	0.53	3.13	0.88
Hillsboro. Glades 9, 12, 13, 14, 18	172	33.98	4.18	9.77	1.29	1.78	0.50	3.03	0.87
Plattin. Glades 15, 16, 19, 20, 21	175	33.65	4.57	9.47	1.38	1.84	0.49	2.89	0.99
Total	744	34.02	4.18	9.63	1.28	1.88	0.53	3.07	0.94

B. Tests of Significance of Differences between Means for Regions

Between Regions	Sepal length, mm.		Sepal width, mm.		Margin width, mm.		Sepal coil, quadrants	
	t	2P	t	2P	t	2P	t	2P
Robertsville and Morse Mill	2.28	0.023	-2.46	0.015	4.15	< 0.0001	1.12	0.26
Morse Mill and Hillsboro	-0.18	0.86	-0.33	0.74	1.64	0.10	1.06	0.29
Hillsboro and Plattin	0.70	0.48	2.08	0.038	-1.04	0.30	1.49	0.14
Robertsville and Hillsboro	1.79	0.075	-2.40	0.017	5.09	< 0.0001	1.65	0.10
Morse Mill and Plattin	0.67	0.50	2.13	0.033	0.55	0.58	2.68	0.0077
Robertsville and Plattin	2.32	0.021	-0.42	0.67	4.32	< 0.0001	2.64	0.0088

other words, whether these samples must be regarded as representing a number of separate populations or as portions of a single statistical population), the data have been subjected to an analysis of variance. The results are presented in Table VI, A. In each of the four flower characters the probability that the 21 series of measurements can be regarded as portions of the same statistical population is very low, clearly beyond the threshold of significance. This excess of variance among glades over that within individual glades is evidence of considerable local differentiation. It may be ascribed to the partial isolation of the glades, which has been discussed above.

It is also of interest to inquire whether these data demonstrate a cline, or regional differentiation of any sort, in any of the characters. In view of the local differentiation, it has not been possible to find evidence of differentiation on a regional scale by examining the means of individual glades, or by studying a series of ideograms, such as Anderson ('36) prepared from his data on *Iris*. The

sampling scheme used in selecting these glades leaves a good deal to be desired when it comes to investigating the question of regional differentiation. Nevertheless, the data for the 21 glades have been combined into four groups as shown in fig. 15 and Table VII, A. The four groups correspond approximately with the Robertsville, Morse Mill, Hillsboro and Plattin regions, described above. An analysis of variance has been carried out for each of the four sepal measurements (Table VI, B). It indicates that the excess of variance from one region to another over that within regions is significant for margin width and sepal coil, perhaps so for sepal width, and not for sepal length. In other words, there appears to be significant regional differentiation in two (or three) of the four measurements.

It is then worth while to compare the means for each of the regions. Means and standard deviations for each of the measurements have been entered in Table VII, A. The t value for the difference between each pair of means has also been determined. In this calculation the variance of the difference has been estimated separately for each pair of means. The probability corresponding to each t value has been found from a table of "Student's" distribution (Table VII, B). In sepal length, the plants from the Robertsville region are perhaps significantly higher than those of the other three regions, while there are no significant differences among the latter. The Robertsville, and perhaps the Plattin, plants have significantly narrower sepals than do those of Morse Mill and Hillsboro. The valvate margin of the sepal is wider in the Robertsville region than in the other three regions, and this difference is highly significant. As in sepal length, the differences in margin width among the Morse Mill, Hillsboro and Plattin regions are not significant. The sepals are most strongly recurved in the Robertsville region and least so around Plattin. The differences in this character between adjacent regions are on the border-line of significance, but the differentiation becomes significant from one end of the range to the other.

In summary, there is significant regional differentiation in each of the four flower measurements. In only one case, sepal coil, can the differentiation be described as a cline, in the sense of a consistent geographical trend. The most striking feature of the differentiation in these characters is the difference between the Robertsville plants and those of the other portions of the population. The same conclusion was drawn above from the analysis of the data on proportion of colored sepal tips (Table IV).

It has been shown above that there is greater differentiation in the flower measurements from one glade to another than on single glades. It may also be inquired whether there is local differentiation from one portion to another of a single glade. To answer this question it is necessary to obtain data on the location on a glade of the plants studied. Such data were not obtained for the plants whose flowers were measured. Laying out quadrats such as those used in population density studies is time-consuming, and it would not have been feasible during the flowering period of *Clematis* to have obtained both flower measurements and accurate locality data for any large number of plants.

On several glades, however, a leaf was collected from each plant plotted during the population density study. The leaves on a single plant vary in size and shape, though those at corresponding positions on different shoots of a single clone are closely similar. To obtain leaves from different plants which would be comparable, one leaf of the pair which subtends the first flower of the plant, or of the most vigorous shoot of a clone, was taken. The leaves of this pair are usually the longest, and comparatively, the widest ones on a shoot. On young sterile plants, however, the apical pair of leaves is usually small, and from such plants a leaf of the largest pair was taken, which was usually at the third or fourth node from the apex. The quadrat in which each leaf was collected was noted on the leaf with wax pencil, or on a small label attached to the leaf with Cellophane tape. Since the leaves are leathery in texture, it was not felt necessary to press them.

Two of the leaf collections have been subjected to measurement and statistical analysis. One of these was obtained over a continuous portion of the large glade at R.6E, T.39N, S.4-O (Glade no. 8 in fig. 2 and Table I). The entire area was laid out in 10-ft. quadrats (fig. 1, B), and a leaf was removed from each plant. Somewhat more than 1,000 leaves were collected. In some cases the label indicating the quadrat in which the leaf was obtained was lost, so that 983 leaves were available for study. Measurements of the dry leaves were made to the nearest mm. with a celluloid rule, some time after collection. Length was measured on the adaxial surface, from the tip to the point of attachment to the stem. Width was measured at the widest place, usually in the proximal half of the leaf. Many of the leaves are not plane, the adaxial surfaces sometimes being markedly concave, and rarely saddle-shaped. In all cases the rule was bent to follow the curvature of the leaf surface. (The author is indebted to John R. Melin, A. S., a U. S. Navy V-12 student, for the measurements, and for assistance in the calculation.)

It may first be inquired whether there are significant differences in the absolute length and width measurements from one portion of the area studied to another. This may be answered, as for the flower measurements discussed above, by carrying out an analysis of variance. For the purpose of the analysis, the area has been subdivided in three ways. The 10-ft. quadrats have first been combined by fours into a total of eighty-eight 20-ft. quadrats, which include an average of 11.17 measured leaves each. The number of leaves in each 20-ft. quadrat varies from 2 to 27 in a non-Poisson manner (see p. 417). Secondly, the 20-ft. quadrats have been combined by fours, with slight irregularities, into a total of twenty-four 40-ft. quadrats, which include an average of 40.96 leaves each, ranging from 9 to 80. The arrangement of the 40-ft. quadrats, and the numbers which have been assigned to them are shown in fig. 1, B. Finally, the entire area has been divided into three strips 80 ft. wide. They consist of the 40-ft. quadrats numbered 1-8, 9-18 and 19-24, and include 435, 435, and 113 leaves respectively. These strips will be referred to as 80-ft. quadrats.

For each of the three schemes of subdivision, calculations have been made of: the sums of squares of the deviations of the length and width of each leaf from

TABLE VIII
MEASUREMENTS OF LEAVES AT R.6E, T.39N, S.4-0
Analysis of Variance and Covariance

A

	Σx^2	Σxy	Σy^2	Degrees of freedom
Within 20-ft. quadrats	249,046.89	186,357.06	199,118.64	895
Among 20-ft. quadrats	77,572.11	71,742.80	76,685.39	87
For variance in length, $n_1 = 87$, $w = 3.20$, $P < 0.0001$				
For variance in width, $n_1 = 87$, $w = 3.96$, $P < 0.0001$				
Within 40-ft. quadrats	279,040.46	210,952.56	226,036.68	959
Among 40-ft. quadrats	47,578.54	47,157.30	49,767.36	23
For variance in length, $n_1 = 23$, $w = 7.11$, $P < 0.0001$				
For variance in width, $n_1 = 23$, $w = 9.18$, $P < 0.0001$				
Within 80-ft. quadrats	314,033.96	244,203.24	260,436.61	980
Among 80-ft. quadrats	12,585.04	13,906.62	15,367.42	2
Total	326,619.00	258,109.86	275,804.03	982
For variance in length, $n_1 = 2$, $w = 19.64$, $P < 0.0001$				
For variance in width, $n_1 = 2$, $w = 28.91$, $P < 0.0001$				

B

	Sum of squares of deviations	Degrees of freedom	Mean square deviation	w	P
Within 20-ft. quadrats:					
Average regression	139,462.41	1	139,462.41		
Regression differences	6,060.80	87	69.66	1.05	0.28
Residuals	53,595.43	807	66.41		
	(199,118.64)	(895)			
Among 20-ft. quadrats:					
Regression of means	66,351.55	1	66,351.55		
Residuals	10,333.84	86	120.16	1.81	< 0.0001
	(76,685.39)	(87)			
Total	275,804.03	982			
Within 40-ft. quadrats:					
Average regression	179,478.60	1	179,478.60		
Regression differences	2,419.41	23	105.19	1.53	0.050
Residuals	64,138.67	935	68.60		
	(226,036.68)	(959)			
Among 40-ft. quadrats:					
Regression of means	46,739.87	1	46,739.87		
Residuals	3,027.48	22	137.61	2.01	0.0034
	(49,767.35)	(23)			
Total	275,804.03	982			
Within 80-ft. quadrats:					
Average regression	189,900.55	1	189,900.55		
Regression differences	1,075.38	2	537.69	7.56	0.0005
Residuals	69,460.68	977	71.10		
	(260,436.61)	(980)			
Among 80-ft. quadrats:					
Regression of means	15,366.98	1	15,366.98		
Residuals	0.44	1	0.44	161.8	0.062
	(15,367.42)	(2)			
Total	275,804.03	982			($n_1 = 977$)

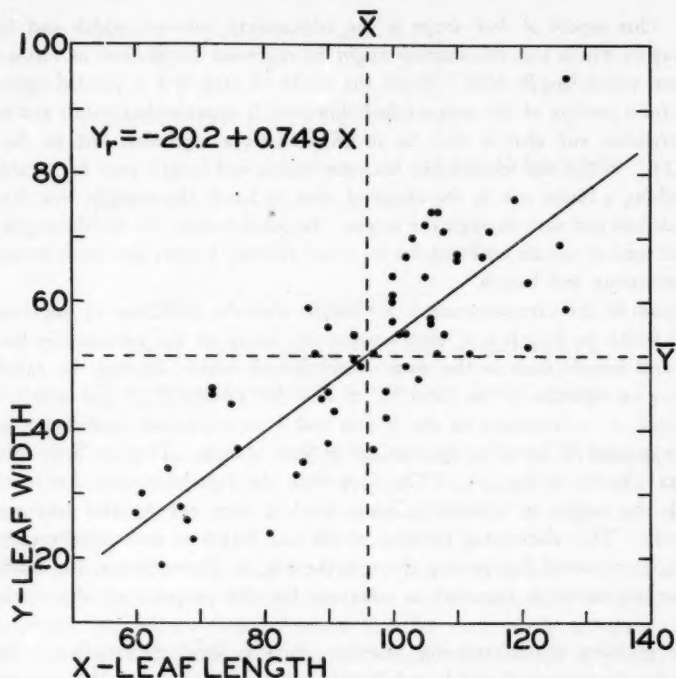


Fig. 17. Length and width measurements of 58 leaves of *C. Fremontii* var. *RiehlII* collected in 40-ft. quadrat no. 8 of fig. 1, B (R.6E, T.39N, S.4-0). Line of regression of width on length has been fitted to the data.

the mean length and width for the quadrat in which the leaf was collected (Table VIII, A, "within quadrats" rows), the sums of squares of deviations of the mean length and width for each quadrat from the over-all mean length and width ("among quadrats" rows), and the sums of squares of deviations of the length and width of each leaf from the over-all means ("total" row). Corresponding sums of products of the length and width deviations have also been determined. By the usual methods of analysis of variance, it is found that there is significantly greater variance among 20-ft. quadrats, 40-ft. quadrats, and 80-ft. quadrats, than within such quadrats, in both the length and width measurements (see the w and P values listed in Table VIII, A).

It is apparent in the field that the size of a leaf is greatly dependent on the general vigor of the plant. Presumably it is strongly influenced by environmental factors, and measurements of absolute length and width cannot be considered of much value in investigating the possibility of genetic differentiation from one portion of a glade to another. It might be supposed that the shape of a leaf is less strongly influenced by environmental variables. It is then of interest to investigate whether these data yield any information about local differentiation in

shape. One aspect of leaf shape is the relationship between width and length. One way in which this relationship might be expressed for a series of leaves is by the mean width:length ratio. When the width of each leaf is plotted against its length for a portion of the area studied, however, it appears that width and length are correlated, and that it may be justified to fit a regression line to the data (fig. 17). While the relationship between width and length may be regarded as approaching a linear one in the range of sizes at hand, the straight line fitted to the data does not pass through the origin. In other words, the width:length ratio does not tend to remain constant for leaves of varying length, but tends to increase with increasing leaf length.

Because of this circumstance, it is thought that the coefficient of linear regression of width on length is a more satisfactory index of the relationship between width and length than is the mean width:length ratio. It may be calculated by fitting an equation of the form $Y_r = a + bX$ (where Y = leaf width, X = leaf length, a = intercept on the Y -axis and b = regression coefficient) to the data for a series of leaves by the method of least squares. This has been done for the data plotted in fig. 17. (The fact that the regression line does not pass through the origin, of course, indicates that it does not fit the data entirely adequately. The relationship between width and length is undoubtedly expressed properly by a curved line passing through the origin. Nevertheless, the coefficient of linear regression is regarded as adequate for the purposes of this statistical study.)

The problem of determining whether there is local differentiation in the relationship between leaf width and length from one portion of this area to another can then be restated as the statistical problem of determining whether the regression coefficients calculated for leaves from different portions of the area are significantly different. This could be done by calculating the coefficients, and applying Student's t test to the differences between pairs. It is possible to do this more efficiently, however, by carrying out an analysis of covariance.

The total variance in width has been divided above (Table VIII, A) into two portions, that within and that among quadrats. The analysis of covariance requires that it be subdivided further. It has been suggested above that there is a significant regression of width on length within at least one of the 40-ft. quadrats (fig. 17). The variance in width within each quadrat could then be subdivided into two portions: the variance of the regression line about the quadrat mean width, and the variance of the individual width measurements about the regression line. The sum of squares of deviations in width can then be written $\Sigma (Y - \bar{Y})^2 = \Sigma (Y - Y_r)^2 + \Sigma (Y_r - \bar{Y})^2$ (where Y = width of an individual leaf, \bar{Y} = mean leaf width for a quadrat, and Y_r = theoretical width for a leaf calculated by substituting its length into the regression equation for the quadrat). If the length and width measurements for a quadrat are put in terms of deviations from the quadrat mean length and width, so that $x = X - \bar{X}$ and $y = Y - \bar{Y}$, the three terms of this equation can be rewritten: $\Sigma (Y - \bar{Y})^2 =$

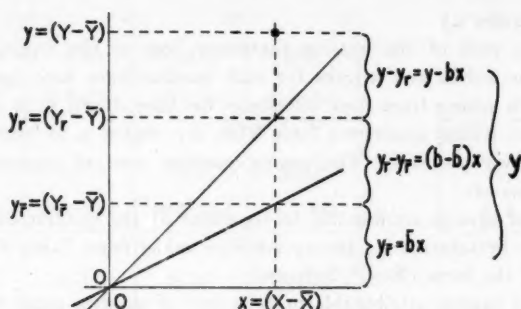


Fig. 18. Diagram to illustrate manner in which deviation in width of a leaf from its quadrat mean has been divided into three portions in the analysis of covariance. Further explanation in text.

Σy^2 , $\Sigma (Y - Y_r)^2 = \Sigma y^2 - (\Sigma xy)^2 / \Sigma x^2$, and $\Sigma (Y_r - Y)^2 = (\Sigma xy)^2 / \Sigma x^2$, as shown in any general treatise on statistics. The equation for the regression line can also be rewritten $y_r = bx$ (where $b = \Sigma xy / \Sigma x^2 =$ regression coefficient for the quadrat).

The regression line for a quadrat, whose equation is written in this way, passes through the origin, that is, through the point which corresponds with the quadrat mean length and width. The regression lines for each of the quadrats can then be visualized as radiating from a common origin. An average regression line for a series of quadrats, which passes through the same origin, can also be considered. Its equation can be written $\bar{y} = \bar{b}x$. In fig. 18 are shown a regression line for one quadrat (lighter line) and the average regression line for a series of quadrats (heavier line). The deviation from the quadrat mean of a single measurement, y , can be seen to be made up of three portions: (1) the theoretical deviation from the quadrat mean, calculated by substituting the length deviation of the leaf into the average regression equation (\bar{y}); (2) the difference between the theoretical deviation calculated from the quadrat regression equation and that calculated from the average regression equation ($y_r - \bar{y}$); and (3) the difference between the actual width deviation and the theoretical deviation calculated from the quadrat regression equation ($y - y_r$). It can be shown that the corresponding sums of squares of deviations in width for all the leaves over a series of quadrats are given by the following formulae:

- (1) Sum of squares attributable to the average regression $= (\Sigma \Sigma xy)^2 / \Sigma \Sigma x^2$;
- (2) Sum of squares attributable to differences between the quadrat regressions and the average regression $= \Sigma [(\Sigma xy)^2 / \Sigma x^2] - (\Sigma \Sigma xy)^2 / \Sigma \Sigma x^2$; and
- (3) Sum of squares attributable to deviations of the separate width measurements from the regression within each quadrat, or "within-quadrats residuals" $= \Sigma \Sigma y^2 - \Sigma [(\Sigma xy)^2 / \Sigma x^2]$. (In each case, the first sign of summation, Σ , indicates summation over a series of quadrats, the second, summations for the series of

leaves within quadrats.)

By centering each of the quadrat regression lines at the origin, differences among the mean widths and lengths for each quadrat have been ignored. The variance in width arising from these differences has been shown to be significantly greater than that within quadrats (Table VIII, A), and it is of interest to subdivide it into two portions. The among-quadrats sum of squares of width deviations consists of:

(4) Sum of squares attributable to regression of the quadrat means, calculated most easily by substituting among-quadrats values from Table VIII, A, into an expression of the form $(\sum xy)^2 / \sum x^2$; and

(5) Sum of squares attributable to deviations of quadrat mean widths from their regression, or "among-quadrats residuals," obtained by subtracting (4) from the among-quadrats sum of squares of deviations in width.

If N is the total number of leaves over the area studied, and k the number of quadrats into which the area is divided, the number of degrees of freedom to be ascribed to each of the five sums of squares is: (1) average regression, 1; (2) regression differences, $k - 1$; (3) within-quadrats residuals, $N - 2k$; (4) regression of means, 1; (5) among-quadrats residuals, $k - 2$; totalling to $N - 1$.

The within-quadrats residual mean square is to be regarded as the "error" mean square, with which other mean squares should be compared. The mean squares of interest in investigating leaf shape differences from one quadrat to another are: that attributable to regression differences (2), and the among-quadrats residual mean square (5). If the population were statistically uniform in the width to length relationship expressed by the regression coefficient, neither of these mean squares should be significantly greater than the within-quadrats residual mean square. (Dr. Donald R. Charles has given generously of his time in developing this scheme of analysis, in clarifying for the author the concepts involved, and in aiding in interpretation of the results of the analysis. The author, however, is responsible for this exposition of the method.)

The analysis of covariance of this leaf collection is summarized in Table VIII, B. A separate analysis has been made for each of the three schemes of subdivision of the area. It will be seen that the regression differences are not statistically significant from one 20-ft. or 40-ft. quadrat to another, but are significant among the 80-ft. quadrats. The among-quadrats residuals are significantly greater than the within-quadrats residuals from one 20-ft. or 40-ft. quadrat to another, but not among the three 80-ft. quadrats. In other words, there is statistically significant local differentiation in leaf shape from one portion of the area to another. This differentiation appears at the 20-ft. and 40-ft. levels of subdivision as significant deviations of the quadrat means from their regression, and at the 80-ft. level as differences among the within-quadrats regressions.

It may then be inquired whether this local differentiation in leaf shape, and in absolute length and width, follows any discernible pattern. For this purpose the outlines of the 40-ft. quadrats shown in fig. 1, B, have been redrawn (fig. 19). Within each quadrat outline have been placed the number of the quadrat (Q),

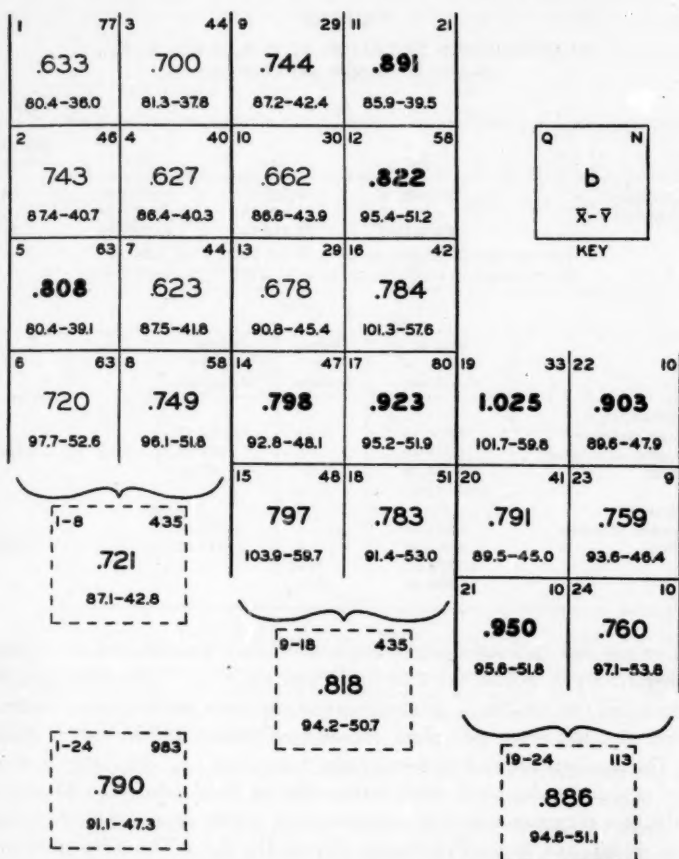


Fig. 19. Collection of leaves of *C. Fremontii* var. *RiehlII* made at R.6E, T.39N, S.4-0. Compare with fig. 1, B. Squares are 40-ft. quadrats. Number in center of each square is coefficient of regression of leaf width on length. Largest coefficients in heavy figures, intermediate coefficients in medium figures, and smallest ones in light figures. Further explanation in text.

the number of leaves measured from it (N), the regression coefficient of width on length for the leaves within the quadrat (b), and the mean length and width of the leaves (\bar{X} and \bar{Y}). When the mean lengths and widths and the regression coefficients are compared, it is seen that the leaves of the upper-left portion of the diagram (embracing, perhaps, the nine quadrats numbered 1, 2, 3, 4, 5, 7, 9, 10 and 11, and a total of 394 leaves) are smaller and relatively narrower, than those from the remainder of the area. This grouping, however, is not without inconsistencies.

TABLE IX
MEASUREMENTS OF LEAVES AT R. 2E, T. 42N, S. 15B
Analysis of Variance and Covariance

A				
	Σx^2	Σxy	Σy^2	Degrees of freedom
Within transects	90,868.35	69,857.32	76,752.97	256
Among transects	6,022.65	5,499.18	7,092.97	8
Total	96,891.00	75,356.50	83,845.94	264
For variance in length, $n_1 = 8$, $w = 2.12$, $P = 0.034$				
For variance in width, $n_1 = 8$, $w = 2.96$, $P = 0.0036$				

B					
	Sum of squares of deviations	Degrees of freedom	Mean square deviation	w	P
Within transects:					
Average regression	53,704.56	1	53,704.56		
Regression differences	1,389.25	8	173.66	1.98	0.080
Residuals	21,659.16	247	87.69		
	(76,752.97)	(256)			
Among transects:					
Regression of means	5,021.13	1	5,021.13		
Residuals	2,071.84	7	295.98	3.38	0.0028
	(7,092.97)	(8)			
Total	83,845.94	264			

The second leaf collection which has been studied was obtained on a glade at R.2E, T.42N, S.15B (Glade no. 2 in fig. 2 and Table I). Nine transects, 10 ft. wide, were laid out at 250-ft. intervals across the glade, as illustrated in fig. 20. Collection of a leaf from each plant encountered yielded 265 leaves for measurement. The numbers for each transect range from 7 to 74. The analysis of variance and covariance described above was applied to these data also. There is significantly greater variance among transects than within in the absolute measurements of width, and perhaps of length (Table IX, A). The differences among the regressions of width on length for separate transects are not significant, but there are significant deviations of the transect means from the among-transects regression (Table IX, B). As before, this can be taken to indicate that there is statistically significant local differentiation in leaf shape from one portion of the glade to another.

In an attempt to determine whether the differentiation on this glade falls into any pattern, the transect regression coefficients have been entered near each transect in fig. 20. When the values of the coefficients are compared, it is seen that they can be arranged in two groups, those of transects 1, 3, 6, 8, and 9 being lower than those of transects 2, 4, 5 and 7. The leaves within each of these two groups of transects are statistically uniform in shape, as shown by an analysis of covariance applied to each. The glade can perhaps be thought of as divided into seven portions which have alternately wider and narrower leaves. These seven

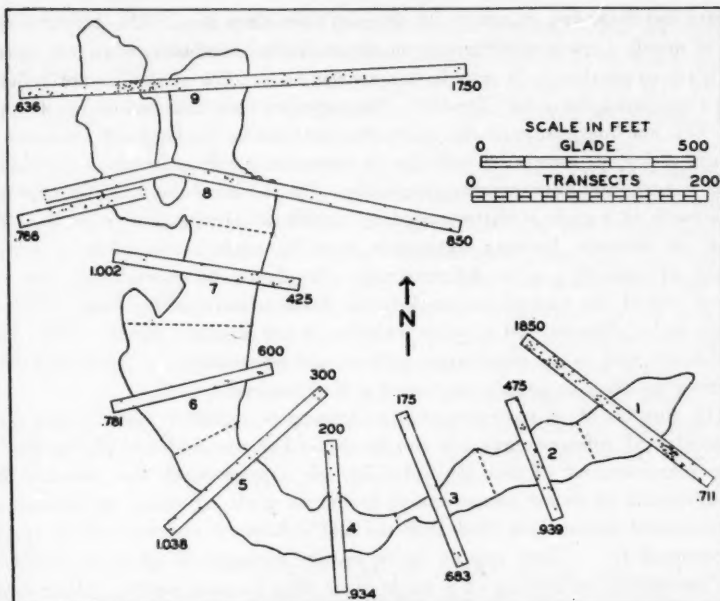


Fig. 20. Glade at R.2E, T.42N, S.15B, showing arrangement of nine transects, and distribution of plants (small dots) within transects. Length and width of a leaf from each plant have been measured. Transects have been enlarged for clarity. The fractional numbers near each transect are coefficients of regression of leaf width on length, the larger numbers have been obtained by multiplying the number of measured leaves from the transect by 25. Further explanation in text.

portions of the glade can perhaps be delimited by lines drawn midway between the transects, as has been done in fig. 20. A crude estimate of the number of plants represented by a transect is obtained by multiplying the number of leaves studied by 25, since the transects are 10 ft. wide and 250 ft. apart. These numbers have also been entered in fig. 20. If this picture of the pattern of differentiation is a true one, the differentiation appears to be effective between groups of plants numbering a few hundred.

An alternative picture of the differentiation pattern on this glade is to regard it as divided into three portions, embracing, respectively, transect 1, with an estimated 1850 plants ($b = 0.711$), transects 2-7, with 2175 plants ($b = 0.987$), and transects 8-9, with 2600 plants ($b = 0.676$). In favor of this grouping is the fact that an analysis of covariance for transects 2-7 shows no significant differentiation in shape among the five transects. If this is the true picture, the differentiation would appear to be effective between groups of plants numbering about two thousand. It does not appear possible from these data to make a choice between the two alternatives.

No information about the factors responsible for the local differentiation in

leaf size and shape can, of course, be obtained from these data. The differentiation may be merely a result of differences in environmental conditions from one portion of a glade to another, or it may have a genetic basis. The glades are not believed to be a uniform habitat for *Clematis*. This topic has been discussed on pp. 418 and 419. This non-uniformity of the glades may give rise to the local differentiation in leaf size and shape merely by inducing environmental fluctuations in a population which is essentially homogeneous genetically. On the other hand, the distribution of *Clematis* on a glade is characterized by significant aggregation (p. 419). The degree of isolation between aggregates may be such as to allow a certain amount of random genetic differentiation. It should be pointed out that, on at least one of the two glades studied, the differentiation in leaf size and shape appears to be effective over a radius including a few hundred plants. Other lines of evidence, such as the distribution pattern, and observations of pollination, have suggested an effective population size of a few hundred.

The question of an environmental, as opposed to a genetic, basis for the demonstrated local differentiation, can also be directed at the results of the analysis of flower measurements (Tables VI and VII). It appears much less probable that the differences in flower measurements from one glade or region to another are environmental fluctuations than that the leaf differences discussed above can be so accounted for. There appears to be greater variation in physical conditions from one portion to another of a single glade than between separate glades taken as wholes. Furthermore, there is undoubtedly a good deal of truth to the systematists' principle that flower and fruit characters are more "stable" than are the characters of vegetative organs such as leaves. Subjective study in the field of variation within and among clones leads one to believe that the principle holds for differences among plants as well as for differences among species.

A number of subjective observations of variation have been made, which consistently point to the existence of a considerable amount of local differentiation. The glade at R.3E, T.42N, S.31D (Glade no. 3 in fig. 15) is small and relatively isolated. It supports 75 to 100 plants. The flowers strike one immediately by their lack of color and unusual proportions. The sepals are longer (one of them measured 51 mm.), and exceed the stamens much more than usual. Perhaps one-third of the plants share these characteristics, and, in other respects as well, show a resemblance which suggests close relationship. At R.2E, T.42N, S.18H (Glade no. 2 in fig. 13) a fairly large glade with a population of 1,000 or 2,000, 15 or so plants were seen which resemble each other in that the sepals are rolled back so as to expose about half the length of the stamen mass. This peculiarity was subsequently seen on a near-by glade, but has not been noticed elsewhere. Two monstrous plants, in which the leaves are irregularly coalesced and incised, and otherwise distorted, were seen among the estimated 600 plants at R.5E, T.40N, S.13A. No teratological specimens have been seen elsewhere, though an occasional ternate shoot has been found in the midst of a decussate-leaved clone. Exploration of the large glade at R.4E, T.41N, S.2F (Glade no. 5, fig. 13)

disclosed only eight plants in a small area at one end. The six which were in flower at the time of the visit were remarkably alike in flower color and form and in general habit. The resemblance suggested that of sibs rather than of portions of a clone. Similar "family resemblances" of plants which are growing fairly close together have been seen less distinctly in many other instances.

Examination of the aerial photograph tracings and field experience both indicate that the glades are, on the whole, smaller and more isolated in the Robertsville region at the northwestern end of the range than they are farther southeast. The difference is more pronounced than the distribution map (fig. 13) suggests. An impression has grown during the field work that this difference in size and degree of isolation of separate colonies is reflected in a difference in the degree of variability in different parts of the range. The plants of the Robertsville region strike one as displaying more variation in the amount of color and pattern of color distribution in the sepals; in the width, texture, and degree of crisping of the expanded margin of the sepal; in the size, shape, and general aspect of the leaves. In general, there is a larger proportion of "queer-looking" plants than among the more uniform population of the Platin region.

SOURCES OF VARIATION

Gene mutation is generally regarded as the ultimate source of evolutionary change, and it would be desirable in studying the evolution of any organism to begin with information about the rate and direction of mutation of its genes. However, such information has been obtained for relatively few genes in a very few organisms which are favorable genetic material. Needless to say, no data whatever on this point are available for *Clematis*, and the plant is not favorable material for genetic study, because of the long period required before it reaches flowering age.

Chromosomal changes such as ploidy, inversion, and translocation have been demonstrated to be responsible for evolutionary change in several organisms. Polyploidy is practically non-existent in the genus *Clematis*. All the reported species are normal diploids ($n = 8$), with the exception of two tetraploid cultivated forms (Meurman and Therman, '39, Gregory, '41). The author has found the haploid number, $n = 8$, in several plants of *C. Fremontii* var. *RiehlII*. Examination in the field of the first division of the microsporocytes of about 75 plants has disclosed no chromatin bridges; in these plants at least, there were no conspicuous inversions.

Hybridization between species and varieties of higher plants is of rather frequent occurrence, and appears to be an important factor in the evolution of many forms. Anderson and Hubricht ('38) have studied a case of introgressive hybridization between two species of *Tradescantia*. Mangelsdorf and Reeves ('39) regard probable hybridization with *Tripsacum* as an important factor in the evolution of maize. Wide crosses are known to occur in the genus *Clematis*. *C. integrifolia*, which is fairly closely related to *C. Fremontii* var. *RiehlII*, has given

rise to *C. Durandi* by a cross with *C. Jackmani*, one of the large-flowered oriental hybrids. A hybrid of *C. integrifolia* with *C. Flammula*, one of the small-, paniculate-flowered species, is also known, and instances of hybridization between other species can be multiplied (Rehder, '40). The fact, then, that *C. Pitcheri*, which is a member of the same section (VIORNA) of the genus as *C. Fremontii* var. *Rieblii*, occurs in the vicinity of the glades makes hybridization between the two species seem at least a possibility. Transfer of pollen between the two species appears possible but must be a rare occurrence. *C. Pitcheri* often occurs in the woods just below a glade, and bumblebees, at least, visit both species (Robertson, '28). However, they are separated by a difference in flowering period. *C. Fremontii* var. *Rieblii* has finished flowering by the second week of May, and *C. Pitcheri* does not come into flower until the middle of June. It continues to flower for some time, and it would probably be in anthesis when *C. Fremontii* var. *Rieblii* flowers sporadically in September.

Five plants have been found which strongly suggest that hybridization does occur. One of the plants (fig. 21, fig. 22, C) appears to be the F_1 progeny of a cross between the two forms. It grows on a glade at R.2E, T.42N, S.14H, about 3.4 mi. southeast of Robertsville. A graded farm-to-market road, surfaced with gravel, crosses the lower edge of this large glade. The supposed hybrid is rooted in the gravel embankment at the down-slope side of the road. The glade above the road is well populated with *C. Fremontii* var. *Rieblii*, a few plants persisting in the gravel at the edges of the road. A number of rather small plants of *C. Pitcheri* occur in the 300-ft. strip of woods between the road and Little Calvey Creek. The site of the hybrid is suggestive, since other species hybrids have often been reported to occur in disturbed habitats.

On the basis of morphological characters, it is impossible to regard the presumed hybrid as a member either of *C. Fremontii* var. *Rieblii* or of *C. Pitcheri*, variable as the latter species is. It appears to show pronounced hybrid vigor. Making allowances for that, it appears roughly intermediate between the two parental forms in the characters which have been examined. It has the ascending habit of *C. Pitcheri*, but the stems are considerably stouter. It appears intermediate in degree of compounding of the leaves between *C. Fremontii* var. *Rieblii*, with simple leaves, and *C. Pitcheri*, whose leaves are compound or decompound, though this character is difficult to evaluate. Its leaflets appear as thick and coriaceous as the leaves of *C. Fremontii* var. *Rieblii*, contrasting with the much thinner leaflets of *C. Pitcheri*. In *C. Fremontii* var. *Rieblii* the flowers are solitary, terminating the vegetative branches; in *C. Pitcheri* single flowers are borne on axillary peduncles, each with one pair of simple floral leaves. In the supposed hybrid both conditions occur (fig. 21). The flowers are intermediate in size between those of the putative parents. The sepals are less recurved, and their valvate margins narrower, than in *C. Fremontii* var. *Rieblii*; in these two characters the plant approaches *C. Pitcheri*. Its flowering period is a week or two later than that of *C. Fremontii* var. *Rieblii*, and earlier than that of *C. Pitcheri*. The clusters of



Fig. 21. Plant which is presumed to be the F_1 progeny of a cross between *C. Fremontii* var. *RiehlII* and *C. Pitcheri*. Note the old flower terminating the primary stem from which sepals and stamens have fallen. Scale in centimeters.

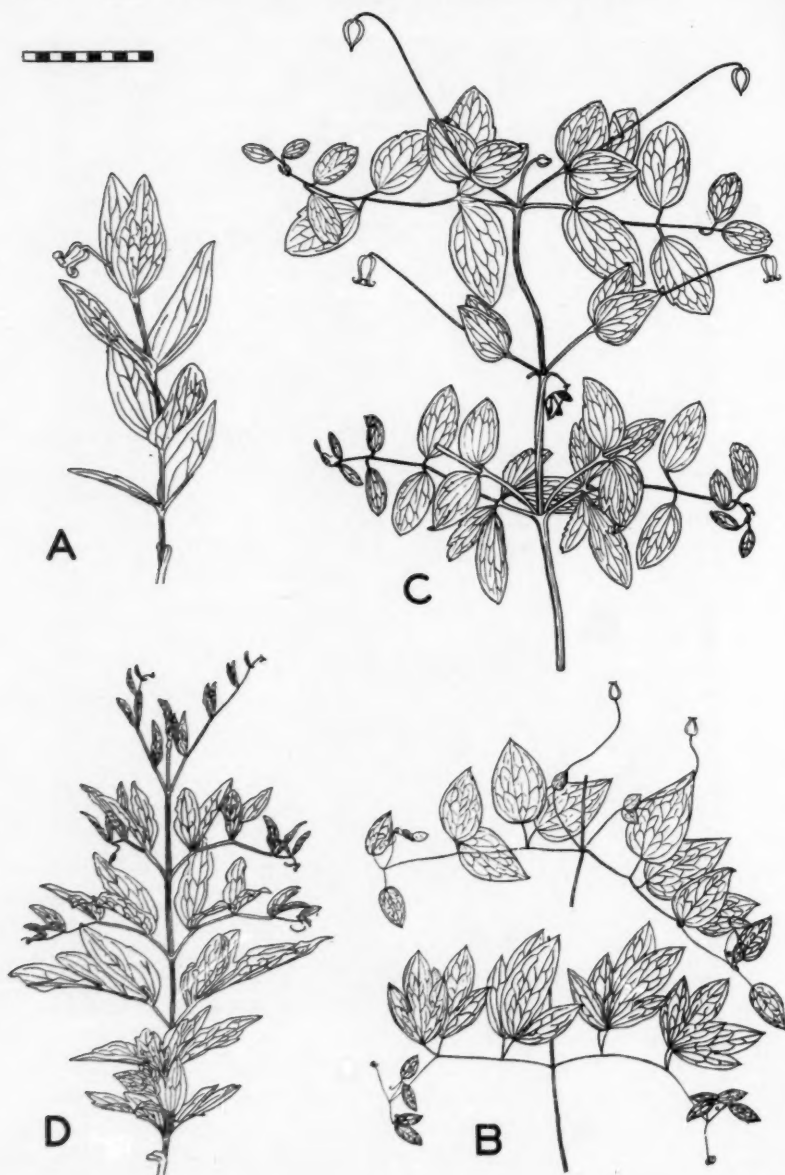


Fig. 22. *C. Fremontii* var. *Rieblüi*, *C. Pitcheri* and two plants which are presumed to be the result of hybridization between them. A. Tracing of a photograph of a young plant of *C. Fremontii* var. *Rieblüi*. B. Tracing of two pressed fragments of a large plant of *C. Pitcheri*. C. Tracing of a portion of a pressed plant, which is presumed to be the F_1 hybrid between *C. Fremontii* var. *Rieblüi* and *C. Pitcheri*. Another shoot of the same clone has been illustrated in fig. 21. D. Tracing of a photograph of a plant which is presumed to have resulted from back-crossing of the F_1 to *C. Fremontii* var. *Rieblüi*. Scale in centimeters.

achenes, and the achenes themselves, are larger than in either of the supposed parents. The achene-tails are naked as in both *C. Fremontii* var. *Rieblii* and *C. Pitcheri*.

It has not been possible to compare the plant with a *C. Fremontii* var. *Rieblii* × *C. Pitcheri* hybrid of known parentage.

The evidence at hand indicates that the supposed hybrid is fertile, though the crucial test of germinating the seeds has not been made. Several full heads of achenes have been seen, and the seeds appear viable on examination. The pollen appears normal in the microspore stage. Five microsporocytes at metaphase I have been analyzed completely. In each of them there appear to be eight normal bivalents. A larger number of cells at this stage have been examined more briefly, and all appear normal. Chromatin bridges have been seen in two microsporocytes out of about 50 at late anaphase I. These observations suggest that there may be one or more inversions differentiating the parents of the supposed hybrid, but that pairing is sufficiently normal to allow formation of good seed.

The supposition that the hybrid between *C. Fremontii* var. *Rieblii* and *C. Pitcheri* is fertile, is consistent with finding of the four other aberrant plants. They all resemble *C. Fremontii* var. *Rieblii* more closely than does the supposed F_1 plant discussed above, but are clearly outside the normal limits of variability of the former. They are not uniform among themselves, and can be arranged in a series according to the degree in which they resemble *C. Fremontii* var. *Rieblii*. Of these four plants, one found at R.2E, T.42N, S.7A and one at R.2E, T.42N, S.15B resemble the F_1 most closely. Next in order is a second plant found at R.2E, T.42N, S.7A (fig. 22, D), and the plant found at R.3E, T.41N, S.1D is nearest to *C. Fremontii* var. *Rieblii*. They suggest a series of backcrosses of the F_1 to *C. Fremontii* var. *Rieblii*.

If these suppositions are correct, some introgression of *C. Pitcheri* genes into the *C. Fremontii* var. *Rieblii* population presumably occurs. It is estimated that the number of plants which have been seen at close enough range to detect such aberrant forms as the five described above is of the order of 10,000. These figures indicate, to a first approximation, the frequency of the presumed introgression. The five plants discussed above appear to have a considerable amount of *C. Pitcheri* germ-plasm; it might also be expected that a larger number of plants would exhibit the presence of a smaller amount. It is not known, of course, in what way small amounts of *C. Pitcheri* germ-plasm might be evidenced. The most striking difference between the two species is the contrast between the simple leaves of *C. Fremontii* var. *Rieblii* and the compound leaves of *C. Pitcheri*. Occasionally a plant is seen which departs from the norm in a coarse toothing of the larger leaves, which are usually entire. It may be that this is evidence of some *C. Pitcheri* genes.

It might also be expected that introgression occurs in the converse direction. The fact that *C. Pitcheri*, *C. Fremontii*, and *C. Fremontii* var. *Rieblii* are the only plants in the genus lacking plumose achene tails is suggestive of exchange of genes between the species over a long period of time.

Another possible source of variability deserves mention. The data on frequency of colored sepal tips (Table IV), the measurements of sepal characters (Table VII), and subjective field observations lead to the conclusion that the plants of the Robertsville region diverge more greatly from the norm for the entire population than do those of the other three regions. The Robertsville glades as a group are relatively isolated, as can be seen by reference to one of the maps, (e. g., fig. 2). It may be that this relative isolation is sufficient to account for the singularity of the Robertsville plants. One is inclined, however, to speculate on the possibility that the population of *C. Fremontii* var. *Rieblii*, limited as it is, may at one time have consisted of two smaller groups. One would suppose that the two groups were centered near Plattin and near Robertsville, since *Clematis* appears most abundant in these regions at present. Their merger may have taken place rather recently, in view of the presumed increase in numbers since white settlement of the Ozarks. This possibility has great evolutionary importance. If the population were at one time divided into two wholly isolated groups, considerable divergence between them would presumably have occurred. The hybridization resulting from their reunion would provide a source of variation of greater magnitude than that provided by gene mutation governed by the statistical mechanism which Wright hypothesizes, and of somewhat different nature than that provided by introgression of *C. Pitcheri* genes.

The variation which is seen in this *Clematis* population could well be the resultant of these three factors. Introgressive hybridization with *C. Pitcheri* is likely. It probably does not occur with great frequency, but genes of adaptive value in the glade habitat may occasionally be introduced into the population by this means. Isolation of the Robertsville region has allowed it to evolve to some extent along its own course, whether one considers the partial isolation of the present, or the possibly complete isolation of some past period. The supposition that some random differentiation of partially isolated groups of plants on separate glades or portions of glades occurs by the mechanism which Wright has described, is consistent with the pattern of distribution of the plant, and with the statistical pattern of variation in several morphological characters. Since it is probable that the effective population size is comparatively large, the fate of individual genes is probably not wholly a random matter, but is under some selective control.

The concept of this *Clematis* population which emerges is not that of an "old" endemic in which evolutionary change has ceased and which is doomed to extinction, though its restricted range may suggest such a picture to some minds. It is rather that of a population which has undergone marked changes in range and in numbers, and which appears to be increasing in numbers at present; one in which evolutionary changes of several sorts are occurring, though perhaps not as rapidly as in many organisms. *C. Fremontii* var. *Rieblii*, because of its presumably low competitive vigor, is probably doomed to restriction to the glade habitat. Its breeding structure is neither that of approximate panmixia which leads to extreme

specialization, nor of extreme restriction in numbers which leads to wholly non-adaptive differentiation. This being so, it may be expected to continue to thrive on the glades, and perhaps to extend its range, though the colonization of new glades will probably be slow.

SUMMARY

Clematis Fremontii var. *Riehlü*, which is wholly restricted to dolomitic barrens, or glades, in an area of about 400 sq. mi. in east-central Missouri, has been studied in the field with particular attention to features of its distribution, biology, and pattern of variation, which are of evolutionary importance.

The population, estimated at 1,500,000, is organized into a hierarchy of natural subdivisions: *regions* of glade concentration; *clusters* of glades; *colonies* of the plant, which correspond approximately with glades; and *aggregates* of a very few, to perhaps a thousand, plants on each glade. There is great inequality in number of plants from one *colony* or *aggregate* to another. Both types of subdivision exhibit partial isolation, of a degree which is regarded as favorable for continuing evolution. The plant appears to be remarkably stable in numbers, but there is indirect evidence that it has increased since the white settlement of the Ozarks.

Inefficient seed dispersal and the longevity of the plants are factors which probably tend to promote a high degree of inbreeding. Counteracting them is the pollination of the plant by wide-ranging insects, which tends to promote cross-breeding.

Statistical study of morphological variation shows significant local differentiation at three levels of the distributional hierarchy: from one region to another, in five flower characters; from one glade to another, in four flower characters; and from one portion of a glade to another, in leaf shape. The most significant feature of the regional differentiation is the singularity of the plants near Robertsville, at the northwestern end of the range.

The pattern of distribution; the biological factors of pollination, seed dispersal and germination, and longevity; and the nature of variation in leaf shape are consistent in suggesting that the effective population size is a few hundred.

There is evidence that introgressive hybridization with *C. Pitcheri* occurs. This, together with differentiation on a regional scale, and local differentiation of a moderately random nature appear to be the most significant evolutionary processes occurring in the population.

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